Chapter 2

Review of Literature
2. REVIEW OF LITERATURE

2.1 INTRODUCTION

Coinfection refers to infection with two or more different disease causing organisms. There is increasing evidence from the molecular to the clinical level that the effects of human immunodeficiency virus (HIV) infection can be modified by coinfection with other viruses. Similarly, HIV can modify the course of prior, simultaneous, or subsequent infection by other viruses; this is particularly seen with the hepatotropic viruses that cause chronic viral infection. The hepatitis viruses A through D are prevalent among patients at risk for human immunodeficiency virus (HIV) infection. Several properties of the hepatitis A through D viruses and the human immunodeficiency virus (HIV) suggest that coinfections are frequent and clinically important. Like HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D (delta) virus (HDV) all can spread parenterally and sexually (Solomon et al., 1988). Hepatitis A virus (HAV) infection is prevalent among homosexual men (Corey et al., 1980) and intravenous drug users, groups at high risk for HIV infection in this world. In addition, HBV, HCV, and HDV can cause chronic infection. Thus, coinfection may be expected to occur relatively frequent. From the literature accumulated over the two decades of the AIDS epidemic, it appears that HIV infection alters the course of hepatitis B, C, and D. Interactions among the hepatotropic viruses and HIV are complex and suggest that mechanisms associated with the immunologic responses to these viruses have not been fully elucidated. It is possible that as patients with AIDS live longer, issues regarding hepatotropic virus infections may become more important.
2.2 DESCRIPTION OF THE AGENTS

2.2.1 Hepatitis B virus

Hepatitis B virus (HBV) is the first human hepatitis virus from which the proteins and genome could be identified and characterized. Before discovery of the viruses, two types of hepatitis (hepatitis A for infectious hepatitis and hepatitis B for serum hepatitis) were differentiated on the basis of transmission routes and other epidemiologic characteristics (Purcell et al., 1993). Hepatitis A virus (HAV) was transmitted by the fecal-oral route, whereas hepatitis B virus (HBV) was transmitted parenterally. In 1965, Blumberg et al. (1965) studied genetic polymorphisms of serum proteins, and discovered a previously unknown antigen in the blood of an Australian aborigine (Australia antigen) that formed a precipitin line with the serum of a multiply transfused hemophiliac (Blumberg et al., 1965). The significance of Australia antigen was soon recognized by its specific association with hepatitis B. By using immune electron microscopic methods, Dane and colleagues in 1970 first described the 42-nm particles that came to be known later as “Dane particles” (Dane DS et al., 1970) and are actually hepatitis B virions. The term Australia antigen was then replaced with hepatitis B surface antigen (HBsAg) to denote its association with the envelope of HBV. In 1973 the viral nature of Dane particles was confirmed by the detection of an endogenous DNA dependent DNA polymerase within their core (Kaplan PM et al., 1973). Subsequently, the HBV genome was found to be a small, circular DNA that was partially double stranded (Robinson et al., 1974).

2.2.1.1 Structure

Hepatitis B virus (HBV) is a member of the family Hepadnaviridae, which includes various closely related viruses that infect birds and mammals. HBV
is an enveloped DNA virus approximately 42 nm in diameter. The viral envelope consists of host-derived lipids and three forms of the viral surface protein. All three surface proteins has a residue N-terminal extension called the preS2 domain. The large surface protein has, in addition, to the pre-S2 domain, a 119 or 108 residue (depending on subtype) N-terminal extension called the pre-S1 domain. Inside the envelope is a nucleocapsid approximately 27 nm in diameter, comprising approximately 200 copies of the viral core protein and probably a single copy of the genome that is approximately 3.2 kb in length. Unique among animal virus families, the viral DNA is a partially double stranded circular molecule, with the viral polymerase protein covalently attached to the 5' end of one strand. Besides virion particles (also known as Dane particles), numerous subviral particles (also called surface antigen particles) circulate in the serum of infected people. In fact, these particles usually greatly outnumber virions (by up to 6 orders magnitude) and constitute the so-called Australia antigen first detected by Blumberg as a marker for HBV infection. Subviral particles contain only lipids and viral surface protein, and are devoid of the nucleocapsid. They also contain little or no large surface protein, which appears to carry the receptor-binding site. Thus subviral particles are not only noninfectious; they are also incapable of interfering with infection by virions.

2.2.1.2 Genotypes, serotypes, subtypes, and determinants

A genotype is generally defined as the genetic constitution of an organism or cell. In an evolutionary context, the term is sometimes more specifically applied to the stable forms into which, for example, viruses develop after undergoing a number of changes over a prolonged time, provided they have been characterized on the basis of their genomic sequences. If they have been characterized serologically, they are often labeled as subtypes or serotypes. It is sometimes presumed that the nature of genotypes or subtypes does not affect a
hepatitis B infection and its clinical course (Grop et al., 1998). This subject, however, remains controversial.

To avoid confusion about the terminology, it is recommended that ‘serotype’ and serological subtype’ be used as synonyms, and that the less specific expression ‘subtype’ on its own be avoided. For similar reasons, the classical definition of the concept ‘genotype’ is advisable, particularly because the term has been applied to HBV strains (E-F) with unknown stability (Magnius et al., 1995).

In case of HBV, a number of serotypes have been described. The existence of the common ‘a’ determinant of HBsAg and the occurrence of atleast two mutually subdominants, d or y, and w or r, resulted in four serotypes, adw, ayw, adr, and ayr. They have a differential geographical distribution and have proven their value as epidemiological markers. The conventionally classified serotypes are sometimes erroneously called genotypes. Genetic analysis has, however, revealed that the four mentioned serotypes do not unequivocally correspond with single genotypes (Grop et al., 1998). At present, six HBV types (A-F) and nine serotypes have been defined. The ‘a’ determinant is of the greatest importance, since it is the dominant epitope cluster of HBV, conferring protection and binding most of the anti-HBs present in hyperimmune serum. Besides, it is highly conserved among the HBV serotypes, and for all of these reasons, HBsAg is a crucial component of current vaccines (Carman et al., 1993 and Halder et al., 1998).

The “a” determinant is part of the major hydrophilic region (MHR) of HBsAg and its tertiary structure has been shown to be important for its antigenicity. In an originally proposed model it consists of 23 aminoacids, situated between positions 124 and 147, and forms two loops protruding from the outer surface of the virus (Carman et al., 1990 and Waters et al., 1992). Most specific antibodies
obtained from vaccines bind to a region composed of nine amino acids, between positions 139 and 147. On the other hand, the ‘a’ determinant has to be considered as a conformational cluster of epitopes extending at least to position 120, because antibody binding around amino acid 122 is affected by variation in the 139-147 region, whereas, antibody binding to amino acids 139-147 is, in turn, influenced by variation between positions 120 and 124. Diagnostic assays designed to detect HBsAg make use of specific antibodies raised against the extended ‘a’ determinant (Carman et al., 1997).

2.2.1.3 HBV mutants

The host-virus relationship is a dynamic and complex interaction with several aspects, including pathogenesis and viral mutagenesis. Generation and/or selection of mutants are a reflection of attempts of the virus to resist immune attacks of the host and to survive. The number and distribution of some HBV mutations in serum from chronic active hepatitis carriers change as a function of time (63,64). Initial point mutations offer a strong advantage if they allow the virus to escape the immune responses of the host. The continued evolution of mutations, however, suggests that a second generation of mutants is selected as a result of the induction of lethal immune reactions against the first generation. The result is that the antigenic complexity of the virus increases over time. Therefore, it becomes more and more difficult for the host to target the virus and to eliminate it by immunological means. In this sense, increasing numbers of mutations during a phase of exacerbation of progressive liver disease might be seen as evidence of adaptive mutation in chronic HBV infection (Chuang et al., 1993).

Mutations of HBV have frequently been described. HBV shows greater mutability than previously expected, because its replication requires reverse
transcription of the RNA pre-genome and this process is known to be susceptible to errors caused by the lack of proof-reading capacity (Yamamoto et al., 1994). This error prone replication leads to $2 \times 10^{-4}$ base substitutions per site per year, i.e. four orders of magnitude more than in other DNA viruses, but less than in RNA viruses (Mimms et al., 1995). Mutants may also arise from recombination between co-infecting strains. HBV mutations are not limited to specific ORFs, and occur in all viral genes and regulatory elements. Mutants have been identified in patients with acute or fulminant as well as chronic HBV infections, and can be catalogued as preC/C gene mutants, presurface/surface (preS/S) gene mutants, and X gene mutants. Patients in whom mutants coexist with wild type viruses are not exceptional. In fact, many infections wherein mutants are involved may be mixed infections of this type, containing different DNA templates that complement each other by trans-activation or by DNA recombination (Tu et al., 1997).

The existence of HBV mutants was first suggested when chronic hepatitis B patients were observed with detectable amounts of HBV-DNA or RNA but without serological indications of active or past infection, as demonstrated by the absence of HbsAg or anti-HBs, respectively. Because HBV infections may persist for years or even decades, mutations may accumulate and become clinically significant. The time factor, the high viral replication activity (with a total release into the peripheral blood of up to $10^{11}$ virus particles per day), and both immune and therapeutic pressures, are decisive determinants in this respect. Besides association with a specific course of the infection, certain mutations may have serious implications at different levels and allow a classification depending on the involved mechanisms. Reduced viral clearance by immune mechanisms or antiviral therapy is called “immune escape” or “therapeutic escape”, respectively. Mutations in the envelope genes can result in a decreased or absent detectability by assays based
upon antibodies to the wild-type virus ('diagnosis escape') or in non-recognition by neutralizing antibodies induced by vaccination ('vaccine escape') (Blum et al., 1995; Nowak et al., 1996 and Blum et al., 1997).

2.2.1.3.1 Precore and core gene mutants

The most frequently observed precore mutation is a G to A transversion at nucleotide 1896. This substitution introduces a translation stop codon (TAG) in the distal precore gene and prevents expression of the preC/C fusion protein that functions as a precursor of HBeAg (Carman et al., 1989). Less common mutations resulting in HBeAg negativity include initiation codon mutations (at positions 1814 or 1815), a nonsense mutation at 1874, a missense mutation at 1862, and frameshift mutation (Kramvis et al., 1997). The serum of patients with these mutations does not contain HbeAg and hepatocytes harbouring this mutant virus do not present HbeAg on their surface. Because HBeAg is an important immunological target, these cells escape killing mediated by the host's immune system. In the early stages of chronic HBV infection, when HBeAg is detectable, the wild virus survives. In the later stages, however, the virus population is reduced in number and, probably as a result of the prolonged interaction with the host's immune mechanisms, mutations affecting HBeAg expression occur. The HBeAg-negative mutants survive, becoming the dominant virus type, and prolong the infection.

The 1896 stop codon mutant is often present in patients with chronic active or inactive hepatitis and in asymptomatic carriers in Mediterranean and Oriental countries (Lee et al., 1996). In contrast, these mutants are rare in North America, Western Europe, and southern Africa (Mangia et al., 1996 and Bowyer et al., 1997). This geographical pattern is determined by the distribution of HBV genotypes. In those regions where genotype A dominates, the 1896 mutant is rare. In
the proximal stem of the RNA encapsidation signal or epsilon (ε), the G residue at 1896 is normally paired with a T(U) at 1858 in non-A genotypes but with a C in the A genotype (Kramvis et al., 1998). A G to A switch at 1986 would result in an unstable base-pairing (A-C) at that position, destabilizing the stem-loop structure of ε and reducing the efficiency of HBV replication (Kramvis et al., 1997). In contrast, in non-A genotypes this mutation creates a Watson-Crick T(U)-A base pair, stabilizing the secondary structure of ε and enhancing viral replication. A mutation at 1899, which may occur in association with the 1896 mutation or other mutations that are associated with HBeAg negativity, is another mutation that improves the stability of ε by providing an additional A-T(U) base pair (Kramvis et al., 1997).

A mutation at position 1862, which occurs in the bulge of ε, has been detected in asymptomatic HBV carriers, and in patients with chronic hepatitis, cirrhosis, hepatocellular carcinoma, or fulminant hepatitis (Kramvis et al., 1997 and Kramvis et al., 1998). This mutation could affect HBeAg expression at two levels. It occurs very close to the signal peptide cleavage site, where the signal peptide is cleaved from the precursor of HBeAg in the endoplasmic reticulum, and might therefore abrogate HBeAg synthesis. Alternatively, this mutation might interfere with reverse transcription of pregenomic RNA. Polymerase (reverse transcriptase) acts as a primer of RNA directed DNA synthesis by binding to the bulge of ε. Although binding of the template to position 1862 is not as crucial as its binding to 1864 and 1865, the 1862 mutation may possibly decrease the efficiency of reverse transcription and hence viral replication. Encapsidation and replication of HBV may also be impaired by mutations in the upper stem and loop of ε (Kramvis et al., 1998).
The core gene contains both humoral and cytotoxic T cell epitopes. Mutation within immunodominant cytotoxic T cell epitopes may be exploited by viruses to evade protective immune responses critical for viral clearance. Deletion of the core gene have been reported in immunocompromised and Oriental patients with chronic hepatitis B or hepatocellular carcinoma (Gunther et al., 1996 and Yuan et al., 1998). These deletions almost always involve loss of B and T cell epitopes and may confer a selective advantage on the virus by evading immune surveillance. Multiple point mutations in the core gene also occur in Oriental and Mediterranean patients with chronic hepatitis B (Ehata et al., 1992 and Carman et al., 1995). Although these mutations may be concentrated at sites with major B and T cell epitopes, it is uncertain how often the function of cytotoxic T cell epitopes is adversely affected by these mutations. Point mutations of the core gene are rarely seen in American or British Caucasian patients with chronic hepatitis B (Gray et al., 1997) suggesting that, in general, the induction of chronic liver disease is not associated with these mutations. Nevertheless, two patients chronically infected with HBV were shown to display mutant epitopes that acted as natural antagonists for T cell antigen receptor recognition and had the capacity to inhibit the cytotoxic T lymphocyte response to the wild-type epitopes (Blum et al., 1997 and Bertoletti et al., 1994). In this way they may have contributed to viral persistence.

In 1989, a precore defective HBV mutant with a G to A substitution at nucleotide 1896 resulting in a TAG stop codon, and failure to produce hepatitis B e antigen (HBeAg) was found in hepatitis B surface antigen (HBsAg) carriers with antibody against HBeAg (anti- HBe) and severe chronic liver disease (Carman et al., 1989).

Over 350 million people worldwide have chronic hepatitis and the majority is in South East Asia and Sub-Saharan Africa. 7 – 30% are infected with
the mutant forms (Huang et al., 2003), with an estimated hepatitis B surface antigen (HBsAg) carrier rate of 4.7% and almost 80% of CLD patients being infected with HBV in India (Thyagarajan SP et al., 1996). The patients with HBeAg negative chronic hepatitis B have a high risk to develop progressive chronic liver disease. High incidence of precore mutants have been reported in patients with sporadic HBsAg positive fulminant hepatitis, from the Far East (Kosaka et al., 1991) and South Mediterranean Countries (Carman et al., 1991).

Furthermore precore mutations have been also detected in fulminant hepatitis type B in Israel (Liang et al., 1991) and in Japan after nosocomial, interspouse or intrafamilial transmission (Carman et al., 1991). Genotypes in Northern Europeans were 60% A, 31% D, in Southern European and Middle Easterners 96% D, in Africans 53% A, 27% D, 20% E, and in East Asians 14% A, 43% B, 43% C (Magnus et al., 1997). The low prevalence of TAG mutation in carriers of Northern European origin has been explained by the presence of cytosine in hepatitis B virus of genotype A, which is the most common genotype in this region (Tong et al., 1993).

Precore mutants are rarely found in carriers of Northern European origin, who usually carry genotype A. Studies in Sweden show C- 1858 strains in 61% of Northern European carriers, 12% of East Asian carriers (Genotype A & C), 3% of Middle Eastern carriers, 50% of African carriers (Genotype A). Thus the emergence of the precore TAG mutation is prevented by C-1858 not only in Northern Europeans but also in a significant proportion of South American, African and South East Asian carriers (Magnus Lindh et al., 1997).

Majority of anti HBe – seropositive HBV carriers in Bulgaria are infected with precore mutant HBV. The precore variant with a stop codon at nucleotide
position 1896 is the dominant virus strain in anti-HBe chronic carriers with severe liver disease. Precore variant virus are associated significantly more often with high viral DNA titres in the serum than infection with the mixed type. Anti-HBe-positive with predominant precore variant infection and high viremia had more severe disease than patients with low viremia and mixed infection with wild type and precore mutant virus (Nikolai et al., 1992).

Precore sequence alters profoundly the properties of the viral core protein. The generation of the p22<sup>c</sup> expressing clone from the p25<sup>c</sup> expressing clone by deleting the precore sequence excludes the possibility that the differences between p25<sup>c</sup> and p22<sup>c</sup> were due to differences in the gene 'C' part (Angela et al., 1986). HBV mutants with precore region defects incapable of directing the synthesis and secretion of HBeAg increasingly prevail in persistent carriers and finally replace the predecessor, wild type HBV after these carriers seroconvert to anti-HBe (Okamoto et al., 1990).

HBV variants bearing mutations in the precore region which abrogate the expression of HBe protein have been readily isolated from chronic hepatitis B patients with circulating anti-HBe antibodies (Tong et al., 1992). The occurrence of hepatitis B virus precore mutants, which arise during spontaneous or interferon – induced seroconversion from HBeAg to anti-HBe, are thought to be selected by immune pressure. A higher response rate to interferon therapy was observed in patients with wild type (75%) than in patients with precore mutant (40%). The data support the hypothesis that precore defective HBV represents viral mutants with an increased capacity to resist exogenous alpha interferon (Zhang et al., 1996).

Severe hepatocellular injury continues to occur during infection with an HBV variant intrinsically incapable of producing HBeAg strongly suggesting that
Continuous synthesis of HBeAg is not required for the progression of HBV induced CLD. (Paul P. Ulrich et al., 1990). Prevalence studies reported from Japan and Israel have shown that the precore TAG mutant was found in 80 – 100% of the patients with fulminant hepatitis B but in none of the patients with acute self-limiting hepatitis B. 7.3% positivity for surface mutants and 9.7% positivity for precore mutants was reported in CLD cases of Eastern India 15.5% positivity of precore mutants and 10.8% positivity of surface mutants was reported in Asian-Indian patients with CLD (Guptan et al., 1996). Patients with precore mutations were always asymptomatic, often presenting with ascites (67%) and jaundice (55%).

The selection of hepatocytes replicating this HBeAg negative virus is probably due to the lysis of the HBeAg positive hepatocytes by anti-HBe. Because the cells infected with the HBe-negative virus express Hbc peptides like hepatocytes infected with HBe-positive virus, emergence of the mutant virus implies absence of cytolytic T cells reactive with HBe protein (Thomas et al., 1995).

Deleted mutants of HBV-DNA in the core region were found to exist in more than half of the patients with chronic hepatitis. Heterogeneity was found in the nucleotide sequences of the precore and core gene including defective mutants in precore with chronic HBV carriers. Products of these variant genes may change the immunological properties of HBeAg (or) HBeAg and subsequently, the clinical course of chronic HBV carriers (Takaji Wakita et al., 1991).

HBeAg negative HBV mutants do not play a predominant etiologic role among North American patients with fulminant hepatitis B. Severe liver damage in chronic hepatitis B infection is related to the clustering missense mutations in codon (48 - 60) and (84 - 101) of core gene. The emergence of precore stop codon mutation and missense mutations around the carboxy-terminal processing site of
Precore/core protein (codons 147-155) may be the adaptive mechanism of hepatitis B virus to decrease production and secretion of viral protein and retain the viral persistence.

Analysis of DNA sequences of HBV genomes isolated from Japanese patients with fulminant hepatitis B, and established that precore mutations are commonly associated with the fulminant hepatitis (Kiyoshi Hasegawa et al., 1991). The precore mutation at the 28th codon, the 29th codon or both was a significant factor in severe forms of hepatitis B in spouses of anti-HBeAg positive carriers of HBV. The HBV population with the 29th codon mutation expanded in the spouse along with a significant rise in the concentration of circulating HBV (Shigeru Yotsumoto et al., 1992).

The analysis of core promoter and precore mutations in HBsAg positive children, revealed a striking presence of both BCP and precore mutants in anti-HBe positive infants with fulminant and severe hepatitis B. The combination of core promoter mutants 1762-T/1764-A and a precore stop codon in HBV genotype D virus strains could be one factor in the pathogenesis of fulminant hepatitis B in infants. There was a high prevalence of precore mutants in HBV sequences with genotype D and a low prevalence in sequences with genotype A. The low prevalence of precore mutants in genotype A suggests that HBeAg positive cases with genotype A have little chance of selecting precore mutants after anti-HBe seroconversion. The distribution of precore mutants between the different genotypes can be explained by the different stabilization energy of each genotype as a result of the introduction of mutation (Rodriguez et al., 1995).

In patients undergoing liver transplantation for HBV related liver disease, infection with precore mutant virus was associated with severe recurrent disease
following transplantation (Angus PW et al., 1995). Reduced immune recognition would allow greater HBV replication within hepatocytes post transplantation. Patients with precore minus HBV infection, HDV replication and spreading might be less efficient because of a hampered helper function provided by the HBV variant (Burnetto et al., 1990).

Cumulative epidemiological and biological observations suggest that the severity of liver damage and outcome of chronic hepatitis B are influenced not solely by the intensity and competence of the hosts antiviral immune response (Ferruccio Bonino et al., 1991). Accumulation of multiple mutations within the precore and core gene region in chronic HBV patients with liver injury and the relative absence of precore and core region mutations in chronic HBV patients without liver injury (Hosono SP et al., 1995). HBeAg-Tg model illustrates that the secretion of a viral protein (ie. HBeAg) which may preferentially deplete inflammatory Th1 cells may represent a viral strategy to promote persistence (David R.Milich et al., 1998).

High prevalence of precore mutant viruses in Anti-HBe carriers with chronic liver disease suggest that monitoring of virus sequence type and DNA level may be of prognostic value for liver disease sequelae. Allele specific PCR is an efficient method for the detection of these viral variants (Lo et al., 1992). Co-existence of serum HBeAg and precore mutants was possibly the result of a release of intracellular core particles from massive necrosis of liver cells and cross reactivity between HBcAg and HBeAg (Hong - Yuan Hsu et al., 1995).

Studies in Hong Kong patients showed that severe disease occurs in patients with serine 15, who do not have large numbers of core amino mutations, indicating that the core variation is not the cause of severe disease but is a result of
immune pressure, which appears less active (or) less focused in those with the serine 15 variant. Precore (or) BCP mutants were not associated with severe liver disease in Western India. Precore mutants appeared to favour an asymptomatic carrier state. HBV DNA levels and Pre-C mutants were identified as independent variables influencing outcome of chronic HBV infection. (Arankalle et al., 2003).

2.2.1.3.2 Core promoter mutations

The core promoter plays a central role in HBV replication and morphogenesis, directing the transcription of both pregenomic RNA and precore mRNA. It overlays the 3' end of the X gene and the 5' end of the preC/C gene. Sequence variation in the core promoter is limited because of its pivotal role in viral replication. The double mutation, A to T transversion at 1762 and G to A transition at 1764, is often present in patients with chronic hepatitis, hepatocellular carcinoma, and fulminant hepatitis, and less often in asymptomatic carriers, immunosuppressed patients, and in carriers without HBV markers (Kramvis et al., 1999). The mutations are accompanied by a reduced level of HBeAg expression resulting from decreased binding of liver enriched factor to this region and the conversion of a nuclear receptor binding site to a hepatocyte nuclear factor 1 binding site. The effect of the double mutation on viral replication is uncertain. A mutation at 1653 in the core upstream regulatory sequence (CURS) is frequently found in patients with fulminant hepatitis (Gunther et al., 1998) and nt 1753-1757 in patients with fulminant hepatitis or hepatocellular carcinoma (Kidd-Ljunggren et al., 1997), both in association with the double mutant. A number of deletions of the core promoter have been described, usually in patients with chronic hepatitis, hepatocellular carcinoma, asymptomatic carriers, and in HBV infection without serological markers. Most deletions result in a frame shift and truncation of the X protein at its C-terminal end, which is essential for its trans-activating function (Kramvis et al., 1999).
Point substitution in the S gene are of particular interest because they affect the immunogenicity of HBsAg, especially the ‘a’ determinant (against which neutralizing antibodies are raised). In an effort to explain the effects that mutations in one region exert, both locally and on linearly distant epitopes, the original two loop model of the ‘a’ determinant (positions 124-147) (Carman et al., 1990), with disulphide bridges between amino acids 124 and 137, has recently been replaced by the cysteine web model of the MHR (positions 100-160 or 169) of the S protein. The current model still takes account of potential disulphide bridges, but additionally supposes cysteines 107, 137, 138, 139, and 149 to be located in a webbed structure in the viral envelope. Two loops (107-137 and 139-147) are external to the virion and probably in opposition, and there is another tight loop between amino acids 121 and 124. The whole MHR is divided into five antigenic regions, named HBs1 (up to position 120), and HBs2 (120-123), HBs3 (124-137), HBs4 (139-147), and HBsS (148-169). There are indications that the loops formed by HBs2 and HBs4, respectively, are spatially close (Carman et al., 1997).

The search for S gene mutants was initiated by the observation that some individuals who are either HBsAg negative/anti-HBc and/or anti-HBs-positive or have no markers for HBV infection, have HBV DNA in their serum. A number of mutants that are not recognized by commercial HBsAg assays have now been documented. Intense interest in the investigation of S gene mutants began with the identification of hepatitis B vaccine escape mutants in infants born to HBeAg-positive mothers who developed breakthrough infections despite having undergone full passive-active immunophylaxis (Carman et al., 1990). HBsAg and anti-HBs circulate concurrently in these babies. The prototype and the most common such escape mutation results in a glycine to arginine switch at amino acids 145 in the
HBs4 region, caused by a guanosine to adenosine substitution at nucleotide position 587 (Carman et al., 1990). This mutant was found in Singapore, Italy, Japan, Taiwan, Indonesia, and Brunei. (Zuckerman et al., 1995, Carman et al., 1995 and Hsu et al., 1997) G145R is stable, because persistence with the same mutant has been shown in children for at least 14 years. Other mutations at codons 123, 124, 126, 129, 133, 142, and 144 occur alone or in combination (65, 105). In a study performed in the USA, of a large number of infants born to HBV carrier mothers, a considerable number acquired chronic infection despite passive-active immunization. In 23% of the infected children, one or more amino acid substitutions were found in the ‘a’ determinant, mostly in positions 142-145. G145R has been proven to be viable, infectious, and pathogenic in chimpanzees (Ogato et al., 1977).

Liver transplantation can be considered for chronic liver disease caused by HBV infection, but is associated with a substantial risk of graft re-infection, graft failure, and death. The conditions for induction of mutations are optimal when administering HBIg after transplantation to prevent re-infection of the donor organ. Mutants emerge in the presence of high levels of antibodies, low quantities of viruses, and a large number of uninfected hepatocytes. The immune pressure exerted by both monoclonal antibodies and HBIg leads to the selection of such escape mutants within HBsAg (McMahon et al., 1992 and Carman et al., 1996). Both deletions and insertions occur. Mutations have often been observed in the MHR section around codon 145 and include the point mutation G145R, which is also the most frequently detected change in HBsAg vaccinated persons (Hawkins et al., 1996).

The question arises whether all mutants of this type are from de novo or from a pool of pre-existing variants that have a selective advantage over the wild-type (Hino et al., 1995 and Hess et al., 1997). G145R has also been demonstrated in
natural isolates and its detectability is poor in many diagnostic assays, especially in the context of the y subtype. Analysis of a random population sample of 2001 HBV carriers without any symptoms in a district of Singapore in 1990-1992 revealed the occurrence of HBsAg mutants in at least 0.8% of the participants (Oon et al., 1998).

The preS1 and preS2 regions, together with the amino acids 100-160 region of the HGS protein, are exposed at the surface of the HBV particles, are highly immunogenic, and are potentially under selective pressure by the system (Mimms et al., 1995). The occurrence of mutations in the preS regions has been demonstrated. Included are deletions of up to one-half of the entire preS1 region (Melegar et al., 1994), deletion of the preS2 translation stop codon and other codons entirely preventing the expression of the preS2 protein (Fernholz et al., 1993), in frame 183 nucleotide deletion, numerous point mutations, and a series of small deletions and insertions. Some deletions also eliminated the recognition sites. In contrast, the hepatocyte-binding site located in the preS1 region is conserved. Deletions of this type potentially lead to impaired virus clearance without affecting HBV attachment to the hepatocytes and subsequent penetration, and therefore could contribute to the development of chronic hepatitis. Additionally, some of these mutants may have been selected following responses aimed at elimination of wild-type HBsAg. Hence, it has been suggested that, if preS determinants are to be included in a future vaccine, this step accompanied by the emergence of pre-S deletion escape mutants in vaccines.

Some mutations in the S and preS regions appear to be associated with the development of human hepatocellular carcinoma (Kim et al., 1996). A transcriptional trans-activator function not present in the intact HBV gene is generated by a mutation in preS/S sequence 3′ truncated during or after integration.
The resulting truncated polypeptides may play a role in hepatocarcinogenesis (Caselmann et al., 1990).

2.2.1.3.4 X Gene mutations

The X gene protein exhibits numerous activities affecting intracellular signal transmission, gene transcription, cell proliferation, DNA repair, and apoptosis (Seto et al., 1990 and Arbuthnot et al., 2000). The most fully studied of these is its promiscuous trans-activating activity. HBX up-regulates a number of viral genes and a wide variety of cellular genes. Its transactivation functions are mediated by activation of transcription factors, modulation of cell signaling pathways, RNA stabilization, and alteration of nucleocytoplasmic translocation. HBX may play a role in persistence of HBV infection and in the development of hepatocellular carcinoma (Takada et al., 1990; Wang et al., 1991 and Arbuthnot et al., 2000).

A number of deletions have been described in the X gene, and they may be associated with a variety of point mutations. An eight-nucleotide deletion at the 3' end of the gene and within the core promoter/enhancer II (CP/ENII) region (position 1770-1777) (Reep et al., 1992 and Uchida et al., 1995) and a 20-nucleotide deletion at 1752-1772 have been described in HBsAg and HBeAg-negative patients. These deletions have been shown to down regulate the preC promoter and this may be the reason for the suppression of HBV protein secretion. Other deletion immediately upstream of direct repeat I (DR I) have been reported in renal dialysis and HBV-vaccinated thalassaemic patients. The absence of conventional serological markers of HBV infection is attributed to these deletions or accompanying mutations in the overlapping CP/ENII region, B cell epitopes DR2, or region coding for the transactivating X protein. A T to C substitution at the extreme 5' end of DR2 was reported in two HbsAg seronegative Japanese patients (Uchida et al., 1995). These
patients also had an eight base pair deletion in the basic core promoter, so the cause of the failure to express HbsAg is difficult to determine. A HBV mutant with fused X-C ORF caused by a single nucleotide insertion in the overlapping region has been identified in a human hepatoma cell line (Kim et al., 1992).

2.2.1.3.5 Polymerase gene mutants

Mutation of the polymerase gene may be associated with resistance to the therapeutic effects of nucleoside analogues and with the viral persistence (Ono-Nita et al., 1999). Lamivudine (2', 3'-dideoxy-3' thiacytidine) is a potent inhibitor of RNA dependent DNA polymerase of HBV, irreversibly blocking reverse transcription and inhibiting viral replication. It thus effectively reduces viral burden in chronic HBV carriers. Long term treatment with lamivudine may, however, lead to resistance as the result of the generation of mutations that disturb the YMDD (tyrosine, methionine, aspartate, aspartate) locus in the C domain of the polymerase gene (Ling et al., 1996). The mutation consists of either a methionine to valine (M552V) or a methionine to isoleucine (M552I) substitution. Both mutations result in amino acid substitutions in codons 195 and 196 in the overlapping S gene. Lamivudine-resistant variants may also have a leucine to methionine (L528M) change in the B domain, occurring often in association with the M552V mutation and rarely with the M552I mutation (Chayama et al., 1998). The L528M substitution has no effect on the aminoacid sequence of the S gene. Replication efficiency of the YMDD mutant is less than that of wild-type virus and, after cessation of treatment, the wild-type virus re-overtakes the mutant. Lamivudine-resistant viruses remain functional and pathogenic.

Famciclovir is the prodrug of penciclovir, an acyclic deoxyguanosine analogue. Penciclovir inhibits DNA-dependent as well as RNA-dependent DNA
polymerase activity. It has a similar therapeutic effects to lamivudine, and is responsible for the emergence of mutants usually involving the B domain of the polymerase gene. However, mutants resistant to famciclovir appear to be less common than those induced by lamivudine. A multi-drug-resistant virus population was created in a liver transplant recipient in whom mutations developed in the polymerase gene as a result of immune prophylaxis and antiviral therapy (DeMan et al., 1998). Nevertheless, viruses that are resistant to lamivudine or famciclovir may remain sensitive to newer nucleoside analogues, such as adefovir and lobucavir.

A mutation of the polymerase gene that might be linked to viral persistence was reported. Polymerase dysfunction, in the form of an inability to package pregenomic RNA into core particles, appeared to result from a single missense mutation in the 5' region of the gene. Trans-complementation in vitro by the full-length wild-type polymerase gene resulted in restoration of the replication competence of the mutant.

Lamivudine inhibits HBV replication in more than 80% of infected patients with or without HIV-1 coinfection. However, emergence of HBV resistance to lamivudine has been widely observed in both populations of patients (Dienstag et al., 1995; Benhamou et al., 1999 and Lai et al., 1998). HBV resistance to lamivudine is seen in 15 to 32% of immunocompetent patients after 1 year of continuous therapy. This rate increases to 38%, 49%, and 67% after 2, 3, and 4 years, respectively (Chang et al., 2000). Emergence of HBV resistance to lamivudine occurs in 50% and 90% of HIV-HBV coinfected patients after 2 and 4 years of therapy, respectively (Benhamou et al., 1999). Patients who develop lamivudine resistance after one year of therapy can show some improvement in liver histology compared with some baseline measurements (Lai et al., 1998). However, the long term effect of lamivudine resistance on subsequent changes in liver histology is not yet known. Cases of HBV resistance to lamivudine after liver
transplantation, resulting in rapid progression to cirrhosis, have been reported (Yoshida et al., 1998). An effective in vivo treatment for lamivudine-resistant HBV strain no longer exists. Therefore additional anti-HBV drugs are needed to treat patients with lamivudine resistant HBV infection and thereby prevent the progression of liver disease in both HIV-1 positive and HIV-1 negative HBV-infected patients.

2.2.2 Hepatitis C virus - Discovery

The term “non-A, non-B” (NANBH) was introduced in the mid-1970 to describe inflammatory liver disease not attributable to infection with HAV or HBV (Purcell et al., 1993). In 1978, the NANBH agent was shown to be transmissible to chimpanzees, as evidenced by the development of liver pathology, including detection of characteristic cytoplasmic tubular structures by electron microscopy (Tabor et al., 1993). Filtration studies showed that the NANBH agent(s) was <80 nm in size and thus likely to be a virus. Sensitivity to chloroform indicated that the NANBH virus is enveloped. The molecular cloning of an NANBH agent from plasma was colleted from a well-characterized, chronically infected chimpanzee with a high infection titer of NANBH agent (Choo et al., 1989). The plasma was subjected to extensive ultra centrifugation to pellet small viruses, and total nucleic acid was extracted from the pellet. The nucleic acid was denatured and cDNA was synthesized by reverse transcription to obtain clones of any viral RNA present. The cDNA was cloned in to λgt11, and one million clones were screened with human NANBH patient serum. A 155-bp clone called 5-1-1 was identified that did not hybridize with the control, human DNA or to DNA derived from two chimpanzees with NANBH. A larger, overlapping, 353-bp clone was then isolated that hybridized to liver and plasma RNA from the original chimpanzee but not the control chimpanzee. These data suggested that the clones were derived from an exogenous RNA molecule associated with NANBH infection. Ribonuclease and hybridization
experiments showed that the clones were derived from a single-stranded plus-sense RNA. This novel RNA virus was subsequently designated hepatitis C virus (HCV). With the subsequent development of antibody-based detection systems, HCV was found to be the major cause of chronic NANBH (Sharara et al., 1996).

2.2.2.1 Description of the agent

Hepatitis C virus (HCV) is a major cause of post-transfusion non-A, non-B hepatitis throughout the world. It is associated with a significant proportion of chronic hepatitis, liver cirrhosis, and has been linked to hepatocellular carcinoma (HCC). About 80% of the infected individuals develop chronic hepatitis, of whom, 10-20% progress to liver cirrhosis with an increased risk of developing HCC. HCV was the first virus discovered by molecular cloning without the use of biological or biophysical methods. This was accomplished by extracting, copying the genome into cDNA, and cloning all the nucleic acids from plasma of a chimpanzee, infected with post-transfusion non-A, non-B hepatitis from contaminated factor XII concentrate (Choo et al., 1989).

2.2.2.2 Structure, Classification and Genome organization

The genome of HCV comprises a positive stranded RNA molecule of about 9600 nucleotides. The genome has a single large open reading frame (ORF) which encodes for a polyprotein made up of ~3000 aminoacids. The genome contains two untranslated regions at the 5' and 3' ends of the genome. The 5' UTR is ~340 nucleotides long, whereas the 3' UTR has a tripartite structure (Kato et al., 1990). The HCV polyprotein is co-and post-translationally cleaved by cellular and viral proteins into ten different products. The structural proteins are situated in the amino terminal of the polyprotein and the non-structural proteins in the carboxy terminal.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Mass (K da)</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>21-22</td>
<td>Nucleocapsid, RNA binding</td>
</tr>
<tr>
<td>E1</td>
<td>31-35</td>
<td>Envelope protein</td>
</tr>
<tr>
<td>E2</td>
<td>70-72</td>
<td>Envelope protein</td>
</tr>
<tr>
<td>P7</td>
<td>7</td>
<td>Unknown</td>
</tr>
<tr>
<td>NS2</td>
<td>21-23</td>
<td>N 2-3 protease component</td>
</tr>
<tr>
<td>NS3</td>
<td>70</td>
<td>NTPase, RNA helicase, Protease</td>
</tr>
<tr>
<td>NS4A</td>
<td>8</td>
<td>NS3/4 protease-cofactor</td>
</tr>
<tr>
<td>NS4B</td>
<td>27</td>
<td>NS5A phosphorylation</td>
</tr>
<tr>
<td>NS5A</td>
<td>56-58</td>
<td>IFN response? Pathogenesis?</td>
</tr>
<tr>
<td>NS5B</td>
<td>65-68</td>
<td>RNA dependent RNA pol.</td>
</tr>
</tbody>
</table>

2.2.2.3 HCV Genotypes

There are 6 major genotypes and more than 65 subtypes prevalent in different parts of the world (Xavier et al., 1998). The genomic sequences of the most distantly related HCV isolates may vary by as much as 35%. Thus, the genetic distance between HCV isolates is comparable with that bound between the genera of enteroviruses and rhinoviruses within the Picornaviridae family and to that between different serotypes of other Flaviviridae (i.e., dengue virus types 1-4). Based on their genetic heterogenicity it is now generally accepted that HCV has evolved into major genetic groups (genotypes) that are further divided into subgroups (sub genotypes or subtypes). Though initially 11 genotypes were defined, now 6 major groups or clades are described. Genotype 1, 2, 4 and 5 belong to clades 1, 2, 4 and 5 respectively; genotype 3 and 10 belong to clade 3; genotype 6-9 and 11 belong to clade 6. These 6 genotypes or clades are further classified into subtypes and more than 100 subtypes have been described so far. The various HCV clades show a definite geographic
distribution. There is a four tiered heterogeneity in HCV genome depending upon sequence similarity. The sequence similarity between members of genotype is 55-72%, between subtypes 75-86% and >88% between quasispecies. Over the past few years, with the development of commercial assays for the identification of HCV genotypes, an impressive number of studies have investigated the molecular epidemiology of HCV infection worldwide and possible effects of HCV genotypes on the pathogenesis and therapeutic outcome of hepatitis C infection.

2.2.2.3a Terminology commonly used in studies related to HCV genomic heterogeneity

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Definition</th>
<th>% Nucleotide* Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Genetic heterogeneity among different HCV isolates</td>
<td>65.7 – 68.9</td>
</tr>
<tr>
<td>Subtype</td>
<td>Closely related isolates within each of the major genotypes</td>
<td>76.9 – 80.1</td>
</tr>
<tr>
<td>Quasispecies</td>
<td>Complex of genetic variants within individual isolates</td>
<td>90.8 – 99</td>
</tr>
</tbody>
</table>

* Nucleotide similarity refers to the nucleotide sequence identities of the full-length sequences of the HCV genome.

However, the different methods used for genotyping and the different criteria for patient inclusion have made comparison of results obtained in different studies difficult. Indeed, although there is consensus that the HCV genotype may influence the outcome of antiviral therapy, no consensus has been reached on other important issues, such as the effects of genotype on transmission, infectivity, pathogenesis and the natural history of the disease.
Methods for HCV Genotyping

2.2.2.4.1 Molecular methods for HCV Genotyping

Because differences in geographical distribution, disease outcome, and response to therapy among HCV genotypes have been suggested, reliable methods for determining the HCV genotype may become an important clinical test. The reference standard and most definitive methods for HCV genotyping is sequencing of a specific PCR-amplified portion of the HCV genome obtained from the patient, followed by phylogenetic analysis of HCV NS5, core, E1, and 5' UTRs. However, direct sequencing is impractical on a large scale because of the complexity of the procedure. Even with the introduction of automated sequencing methods that do not require radioactive isotopes, only a few laboratories are equipped to perform these procedures on a regular basis. Finally, sequencing of amplified DNA does not usually identify mixed infections with two different genotypes.

Other methods that have been reported depend mainly on the amplification of HCV-RNA from clinical specimens, followed by either reamplification with type-specific primers or hybridization with type-specific probes (Okamoto et al., 1992; Li et al., 1994 and Qu et al., 1994) or by digestion of PCR products with restriction endonucleases that recognize genotype specific cleavage site (McOmish et al., 1993). HCV genotyping by using type-specific primers was first performed by Okamoto et al. (1992) and they used primers specific for the core region. This method lacked sensitivity and specificity (Xavier et al., 1998). Without modification, this method was able to detect subtypes 1a, 1b, 1c, 2a, 2b, and 3a. However, modifications have been introduced to improve the sensitivity and specificity of this method (Okamoto et al., 1996), but more studies are required before the efficiency of this genotyping method can be compared with that of other methods. Several DNA hybridization assays for HCV genotyping have been
described. A commercial kit (Inno Lipa) for HCV genotyping has been introduced in Europe by Innogenetics (Zwijndrecht, Belgium) and is based on hybridization of 5' UTR amplification products with genotype-specific probes (Stuyver et al., 1993). Although the initial version of Inno Lipa had lower sensitivity, the newer version is capable of discriminating among HCV subtypes 1a, 1b, 2a, to 2c, 3a, to 3c, 4a, to 4h, 5a, and 6a (Maertens et al., 1997). It has been shown that genotyping methods using 5' UTR, including Inno Lipa, may not distinguish subtype 1a, from 1b in 5 to 10% of cases and also may not distinguish between subtypes 2a and 2c (Smith et al., 1995).

In the restriction fragment length polymorphism method (RFLP), a PCR amplified DNA fragment is digested into fragments with different lengths by enzymes (restriction end nucleases) that recognize cleavage site specific for each genotype (Xavier et al., 1998).

Although all these methods are able to identify correctly the major genotypic groups, only direct nucleotide sequencing is efficient in discriminating among subtypes (Bukh et al., 1992 and Simmonds et al., 1996). Moreover, all of these PCR-based methods have the shortcomings and advantages of PCR. They are expensive and time consuming and require specialized facilities to ensure accurate results and prevent contamination. Their reliability may further be compromised if viral RNA is lost in the serum or plasma through storage or improper laboratory handling or if it is absent from the circulation during sample collection. The advantages of PCR-based methods include reliability if performed accurately and the ability to obtain information relevant to the molecular pathogenesis of HCV.
More recently, investigators identified genotype-specific antibodies that could be used as indirect markers for the HCV genotype (serotyping or serologic genotyping) (Machida et al., 1992; Simmonds et al., 1993 and Mondelli et al., 1994). Serologic genotyping is suitable for large epidemiologic studies owing to advantages including the low risk for contamination and the simplicity of the assay. However, serologic typing seems to lack specificity and sensitivity, which limits its usefulness.

Two commercially available serologic genotyping assays have been introduced over the past 3 to 4 years. The RIBA SIA was introduced by Chiron Corp. and contained five different serotype-specific peptide sequences taken from the core region of the HCV genomes for genotypes 1, 2, and 3 (Dixit et al., 1995). The second serologic genotyping assay is the Murex HCV serotyping enzyme immune assay (Murex Diagnostics Ltd.), which is based on the detection of genotype specific antibodies directed to epitopes encoded by the NS4 region of the genomes for genotypes 1 through 6. These two assays have been compared and showed a concordance rate of more than 96% for genotypes 1, 2, and 3 (Gish et al., 1997).

A recent study by Beld et al. (1998) showed high reliability of HCV serotyping by the RIBA SIA (Chiron Corp. Emeryville, Calif.) in immunocompetent individuals infected with genotype 1a. However, the assay had low sensitivity in samples containing genotype 3a or in samples from patients coinfectected with HIV. These findings suggest that the use of this assay may be limited at this time, particularly in geographic regions where genotype 1a is not prevalent. Similarly, Songsivilai et al. (1998) showed that serotyping had poor sensitivity for samples
from patients infected with HCV genotype 6. Unlike the two previous studies, a study conducted in the United States reported high concordance between serologic genotyping and molecular genotyping assays (Gish et al., 1997). These findings suggest variation in the reliability of these assays based on the distribution of HCV genotypes in a specific geographic area.

The choice of typing method for HCV should be based on the expertise in a specific laboratory or institution and the goal of typing. To identify all subtypes and to identify novel sequences if present, PCR amplification followed by sequencing should be the method of choice. However, the goal in treatment trials is frequently to separate patients infected with genotype 1 from those infected with other genotypes—a task that could be done adequately by any of the methods mentioned.

2.2.2.5 Geographic distribution of HCV genotypes

At least six major genotypes of HCV, each comprising multiple subtypes, have been identified worldwide (Zein et al., 1996). Substantial regional differences appear to exist in the distribution of HCV genotypes. Although HCV genotypes 1,2 and 3 appear to have a worldwide distribution, their relative prevalence varies from one geographic area to another. HCV subtypes 1a and 1b are the most common genotypes in the United States (Zein et al., 1996). These subtypes are also predominant in Europe. In Japan, subtype 1b is responsible for up to 73% of cases of HCV infection (Takada et al., 1993). Although HCV subtypes 2a and 2b are relatively common in North America, Europe, and Japan, subtype 2c is found commonly in Northern Italy. HCV genotype 3a is particularly prevalent in intravenous drug abusers in Europe and the United States. HCV genotype 4 appears to be prevalent in North America and Middle East (Chamberlain et al., 1997) and
genotypes 1b and 3 are prevalent in India (Panigrahi et al., 1996). Genotypes 5 and 6 seem to be confined to South Africa and Hong Kong respectively (Cha et al., 1992 and Simmonds et al., 1993). HCV genotypes 7, 8 and 9 have been identified only in Vietnamese patients (Tokita et al., 1994) and genotypes 10 and 11 were identified from Indonesian patients (Tokita et al., 1996).

The geographic distribution and diversity of HCV genotypes may provide clues about the historical origin of HCV (Smith et al., 1997). The presence of numerous subtypes of each HCV genotype in some regions of the world, such as Africa and Southeast Asia, may suggest that HCV has been endemic for a long time. Conversely, the limited diversity of subtypes observed in the United States and Europe could be related to the recent introduction of these viruses from areas of endemic infection.

2.2.2.6 Clinical Relevance of HCV Genotypes

Although the impact of HCV heterogeneity and genotypes on the day-to-day clinical management of chronic HCV infection has not been established, its role as an epidemiologic marker has been clearly shown. Furthermore, the sensitivity and specificity of serologic and virologic assays for the detection of HCV may be influenced by the heterogeneity of HCV. However, the outcome of HCV infection, and the response to interferon therapy are much less well understood than their role as an epidemiologic marker. The study of these issues have been hampered by the long natural history of HCV infection and the lack of information about the exact time of exposure to the infection. The following subsections in this section specifically address these issues on the basis of the information available.
Because of geographic clustering of distinct HCV genotypes, genotyping may be a useful tool for tracing the source of an HCV outbreak in a given population. Genotyping has been used to trace the sources of outbreaks of HCV in Germany. More recently, genotyping and molecular characterization of HCV isolates provided evidence for a patient-to-patient transmission of HCV during colonoscopy (Bronowicki et al., 1997). The index case as well as the two other infected patients had HCV genotype 1b. Nucleotide sequencing of the NS3 region showed that three patients had the same isolate (100% homology), strongly suggesting common source of infection.

Suspected non-conventional routes of HCV transmission could also be investigated by molecular analysis of HCV strain from different persons. These include the vertical and sexual routes of transmission. A single predominant HCV variant was a reported transmission to an infant born to a mother with multiple variants (Weiner et al., 1993). A specific 12-nucleotide insertion in the E2 hypervariable region of the HCV genome was noted in the vertically transmitted sequence of an infant born to a mother infected with two different genotypes, each composed of multiple heterogeneous sequences (Aizaki et al., 1996). These data may suggest a potential role of HCV heterogeneity and genotypes in mother to infant transmission of HCV (Zein et al., 1997).

Reports on the sexual transmission of HCV infection are conflicting. The detection of anti-HCV positivity ranged from 0% in partners of transfusion-associated hepatitis patients (Everhart et al., 1990) to 8% in male homosexuals (Esteban et al., 1989) and 5% in household contacts. A possible explanation is that sexual transmission occurs only in association specific HCV genotypes or in the
presence of specific mutations along the HCV genome. As with vertical transmission, samples from patients with suspected sexual transmission of HCV have undergone nucleotide sequence analysis to confirm the similarity of sequences obtained from sexual partners and thus the common origin of these HCV strains (Chayama et al., 1995).

Although Zein et al. (1996) found no association between HCV genotypes and the mode of HCV acquisition in their population, others have provided evidence for such an association (Berg et al., 1997) between these. It has been suggested that genotype 3a and 1a are closely associated with intravenous drug use and that genotype 1b is seen more often in patients who acquired HCV through blood transfusion. This information may be useful in tracing sources of HCV epidemics.

2.2.2.6.2 Development of diagnostic assays

Cloning of the HCV genome led to the identification of 5-1-1 proteins that was reactive with sera obtained from patients with non-A, non-B hepatitis. Using 5-1-1 as a hybridization probe, the recombinant antigen c100 was expressed in yeast and eventually was used to develop the first screening assay. The first generation HCV antibody test approved by the Food and Drug Administration became commercially available in 1990 and was widely used. This was an ELISA that incorporated the c100 epitope from the NS4 region. Because of the high level of false positivity, SIAs were introduced as a supplemental test in patients with positive ELISA results.

As more reactive recombinant antigens were identified from conserved regions of the HCV genome, newer serologic assays (second and third generation) were introduced. With approved sensitivity and specificity, the newer assays quickly
replaced the first-generation assays. These second-generation assays included the ELISA-2 and SIA-2, which were approved by the Food and Drug Administration in 1992 and 1993, respectively. In addition to the 5-1-1 and c100-3 antigens, these assays incorporated the c22-3 antigen derived from the core region of the HCV genome and the c33c antigen derived from the structural region NS3. Third generation assays (ELISA-3 and SIA-3) were introduced in Europe more than 6 years ago. In these assays, a recombinant NS5 antigen has been added to the four antigens in the second-generation assays. These third generation assays have higher sensitivities and specificity than second-generation assays and are much less strongly influenced by the infecting genotypes (Makris et al., 1995 and Kao et al., 1996).

The detection of HCV-RNA by reverse transcriptase PCR has become essential for the diagnosis of HCV infection and for the selection of patients before therapy. The main advantages of reverse transcriptase PCR include early diagnosis after acute infection and detection of viremia in selected patients (those with indeterminate antibody results and immunosuppressed patients). The sensitivity of PCR for HCV-RNA detection may vary according to the choice of primers and handling of pre-extraction samples (Bukh et al., 1992 and Busch et al., 1992). Most laboratories use primers specific for the 5' UTR of the HCV genome because it represents the most highly conserved region among HCV genotypes. Assays for the quantitation of HCV viremia levels in serum (HCV-RNA titer) have been developed and shown to be valuable in the selection of patients for interferon treatment and in the assessment of response during therapy (Peignoux et al., 1995). Two different methods have commonly been used for the quantitation of HCV RNA and have become commercially available. The first was developed by Roche Diagnostics Systems (Branch-burg, N.J.) and is based on a competitive PCR assay for HCV
RNA quantitation (Roche Monitor assay). The second method is based on the co-amplification of synthetically mutated target RNA (branched DNA (bDNA) assay: Quantiplex (Chiron corp.). The quantitative results of HCV-RNA detected by both methods are reliable and reproducible (Lu et al., 1998).

Earlier studies have suggested a difference in the efficiency of the bDNA assay (Quantiplex HCV-RNA 2.0) that incorporated a set of oligonucleotide probes to enhance the efficiency of binding to genotypic variants of HCV (Deimer et al., 1996). The new assay was highly sensitive and virtually unaffected by HCV genotypes. A similar variation in the efficiency of detection of different HCV genotypes was recently observed for the Roche Monitor assay (Hawkins et al., 1997). These genotype-dependent differences in the efficiency of assays to accurately quantify HCV-RNA may have to be considered if clinical decisions are based on the results obtained.

2.2.2.6.3 Outcome of Acute HCV infection

After initial exposure to HCV, the infection fails to resolve in the majority of patients (80%) who become chronically infected. The ability to evolve into chronic disease associated with liver damage is by far the most striking feature of HCV. The spontaneous clearance of HCV following acute infection in a small proportion of patients has been the focus of intense investigation. It has been proposed that differences in the host cellular (Missale et al., 1996) or humoral immune responses to HCV are important in spontaneous clearance, but these proposals remain unproved.

Amoroso et al. (1998) specifically investigated the role of HCV genotypes in persistent of HCV infection following an acute exposure. The rate of evolution to chronicity after acute exposure to HCV was 92% in patients exposed to
HCV genotype 1b infection, compared with 33% to 50% in patients exposed to other genotypes. These data provided evidence that viral factors, including the HCV genotypes, may potentially play an important role in the development of chronic infection following acute exposure to HCV.

2.2.2.6.4 Progression of Liver Disease

The role of HCV genotypes in the progression of liver disease is one of the most controversial areas of HCV research. There appears to be significant biologic variation in HCV disease expression in the host over the length of the infection (typically the life of the patient). Currently, investigators are divided into those who strongly believe in differences in pathogenicity among genotypes and those who do not. Conclusions have been derived from indirect evidence, because conducting accurate investigation to answer these questions has been difficult. The role of genotypes as an independent factor in the progression of liver disease cannot be separated from the roles of other cofactors such as viral load, alcohol intake, and the length of time of HCV infection. Patients may not provide accurate information about drug use or the amount of alcohol intake. Therefore, the time of HCV acquisition often is not known. Because of the overall slow progression of liver disease in HCV-infected patients, prospective studies frequently are not possible.

In patients with chronic HCV, infection with genotype 1b is associated with a more severe liver disease and a more aggressive course than in infection with other HCV genotypes. Similar to others (Pistello et al., 1994 and Zein et al., 1995 & 1996) found that HCV genotype 1b was significantly more prevalent among patients with liver cirrhosis and those with decompensated liver disease requiring liver transplantation than among those with chronic active hepatitis C. Although this is indirect evidence, it suggests an association between HCV genotype 1b. There is
compelling evidence that hepatocellular carcinoma occurs more frequently or emerges earlier among HCV-infected Japanese patients (Takahashi et al., 1993 and Yano et al., 1993) than among HCV carriers in Western countries (Hopf et al., 1990). Because HCV genotype 1b is more common in Japan than Europe or the United states, the hypothesis relating to genotype is attractive and appears to explain these differences. Furthermore, HCV genotype 1b was present in most of the patients with HCV-associated hepatocellular carcinoma studied by Zein et al., (1996). Similarly, Reid et al. (1994) determined the HCV genotypes in 28 patients with hepatocellular carcinoma and found that 19 (68%) were infected with HCV genotype 1b and the rest were infected with a mixture of HCV genotypes that always included genotype 1b.

Some reports refute the associations mentioned above (Yamada et al., 1994; Simmonds et al., 1995; Lau et al., 1996; Benvegnu et al., 1997 and Naoumov et al., 1997). A possible and simple explanation may reconcile these reported discrepancies. Zein et al. (1996) found that patients infected with HCV genotype 1b were older than those infected with other genotype and that genotype 1b may have been present before the other genotypes. Thus, patients infected with genotype 1b may have been infected for a longer time. HCV genotype 1a was introduced into the United States in the late 1950s and then became the most prevalent genotype in the United States. It was not until the 1960s and 1970s that genotype 2 and genotype 3 and 4, respectively, were introduced in the United States. After accounting for differences in the duration of HCV infection, HCV genotypes were distributed equally among patients with mild or advanced liver disease. Similar observations have been made in France and Spain (Pol et al., 1995). According to this explanation, HCV genotype 1b is a marker for more severe HCV associated liver disease, because it reflects a longer time of infection rather than a more aggressive
form of hepatitis C. Zein et al. (1995) and Gorden et al. (1997) reported that in liver transplant recipients, HCV genotype 1b is associated with earlier recurrence and more severe hepatitis than are other genotypes. Although others have reported similar findings, some authors have suggested that there is no association between genotype and HCV recurrence after transplantation (Zhou et al., 1996 and Boker et al., 1997). The difference in the duration of infection that may have been a factor in non-transplant-associated HCV patients is not likely to explain the discrepancies in the literature about post transplantation HCV.

### 2.2.2.6.5 Response to Interferon Therapy

Since the discovery of HCV, considerable effort has been devoted to defining the factors that may be important in predicting the long-term response to interferon therapy (Zein et al., 1995). The interferon dose, duration of treatment, viral RNA level (Weiland et al., 1993), and liver histology (Lin et al., 1991) all seem to play a role in predicting response. It has been suggested that patients infected with HCV genotypes 1b and, to a lesser degree, 1a are less likely to have a favorable response to interferon treatment than are those infected with genotype 2 or 3. Zein et al. (1996) reported a complete biochemical response at the end of 6 months of treatment with interferon in 60 to 70% of patients infected with HCV genotype 2 and in 10 to 15% of those infected with genotype 1. More recent treatment trials using interferon-naive patients with chronic HCV (McHutchison et al., 1998) or in patients in whom previous interferon treatment failed (Smith et al., 1997) showed higher rates of sustained response to therapy in patients with HCV genotypes other than 1. An “interferon-sensitive” region in the nonstructural portion of the HCV genome has been identified in Japanese patients infected with genotype 1b (Enomoto et al., 1995). However, studies from the United States and Europe failed to confirm these findings (Odeberg et al., 1998).
2.2.2.7 HCV Genotypes in India

The genotype 1b and 3 are predominantly found in India. It is important to note that from neighboring countries such as Nepal and Thailand, HCV type 3 variants have been described. However, the isolates from Southern India resemble more closely the sequences from Indonesia (Valliammai et al., 1995). This follows the pattern of population migration and trade route of two parts of the Indian subcontinent. A new subtype of HCV from this region, provisionally designated as 3g (Panigrahi et al., 1996).

2.2.3 Human Immunodeficiency Virus

Human immunodeficiency virus (HIV), the etiologic agent of AIDS, is estimated to have infected over 60 million people. Infection with HIV type 1 (HIV-1) is prevalent throughout the world and is characterized by a progressive deterioration of the immune system which is almost uniformly fatal if untreated. This pandemic is now the fourth leading cause of mortality worldwide and over 95% of these deaths have occurred among young adults in the developing world. Although the first known case of HIV infection of a human dates to 1959 (Zhu et al., 1998), cases of AIDS were first recognized clinically in 1981. The syndrome was identified based on unexpected clusters of diseases including Kaposi’s sarcoma and Pneumocystis pneumonia in young homosexual men (Masur et al., 1981). These patients were noted to have immunodeficiency due to depletion of CD4 positive helper T cells (Gottlieb et al., 1981). AIDS cases were subsequently reported in intravenous drug users, hemophiliacs, and infants born to mothers with AIDS, suggesting blood-borne as well as sexually transmitted pathogen. In 1983, HIV-1 was isolated (Barre-Sinoussi et al., 1983) and this novel human retrovirus was proposed as the cause of AIDS. During the next several years, an antibody test was
Developed to detect the infection, the nucleotide sequence of the genome of HIV-1 was determined (Sanchez-Pescador et al., 1985), and the first antiretroviral drug, the nucleoside analogue zidovudine, was shown to have activity in vitro and in patients (Mitsuya et al., 1985). Since then, enormous progress in both basic and clinical research has provided the clinician in the developed world with the tools to suppress viral replication to a degree sufficient to prevent or reverse the immunological and clinical sequelae of HIV infection. Nevertheless, neither definitive therapy for this disease nor a vaccine to stop the spread of the pandemic is available.

2.2.3.1 Virology - Classification

HIV-1 and HIV-2 are enveloped RNA viruses belonging to the *Lentivirus* genus in the family *Retroviridae* (Chiu et al., 1985). These viruses reverse transcribe their genomes to form complementary DNA (cDNA), which integrates into the genomic DNA of the host as a provirus. Lentiviruses are characterized by cytopathogenicity in vitro, lack of oncogenicity, the establishment of chronic infections, and relatively slow rates of development of disease. HIV-1 and HIV-2 are related to the nonhuman primate lentivirus SIV (Desrosiers et al., 1989). SIV strains have been found in 26 species of non-human African primates, including African green monkeys (SIVagm) and sooty mangabeys (SIVsm) (Hahn et al., 2000). New SIVs are being identified as additional African monkeys are examined. In their natural hosts, these viruses are not pathogenic. However, introduction of SIVsm or SIVagm into *Rhesus macaques*, an Asian monkey, resulted in an AIDS-like illness, similar to that caused by HIV infection in human (Letvin et al., 1985).

2.2.3.2 Genotypes, Serotypes, and Antigenicity

There are three distinct genetic groups of HIV-1, designated M, O and N. Group M viruses dominate the pandemic. This group is divided into genetic
subtypes or clades (A through D, F through H, J and K) based on nucleotide sequence relatedness. Certain viral isolates appear to be recombinants containing sequences from more than one subtype. These are designated CRF (for “circulating recombinant form”) (Robertson et al., 2000). For example, the former subtype E, prevalent in Thailand, is now believed to be a recombinant between subtypes A and E (in fact, no full length subtype E viruses have been described), and its proposed designation is CRFO1-AE. Mosaic viruses that contain parts resembling four or more subtypes are given the suffix cpx for complex; for example, the former subtype I has been given the designation CRF04-cpx to indicate that it is a complex circulating recombinant form. There are no clearly defined HIV-1 serotypes.

The humoral immune response relevant to immunity concerns the viral envelope glycoprotein (Env), which is exposed on the surfaces of infected cells and virions. The HIV-1 Env is composed of a surface domain (gp120) and a transmembrane domain (gp41), which are noncovalently associated and forms trimeric spikes. The peptide sequence of Env contains conserved (C) and variable (V) regions. Env has several features that render it as suboptimal antigen and immunogen (Kwong et al., 1998). The surface of gp120 is heavily glycosylated, and this glycosylation reduces the antigenicity of Env, presumably by allowing the molecule to appear to the immune system as “self”. Regions of Env that are conserved because of their involvement in the binding interactions with cellular molecules required for infectivity are poorly accessible to antibodies. For example, the binding site on gp120 for CD4, the primary cellular receptor for the primate immunodeficiency viruses, is recessed and surrounded by variable glycosylated regions. Similarly, the binding site for the chemokine receptors, the so-called coreceptors for these viruses, is masked by the variable loops V2 and V3 until CD4 is engaged (Rizzuto et al., 1998).
2.2.3.3 Composition of Virus

2.2.3.3.1 Virion Morphology, Structure, Size and Genomic Organisation

The HIV-1 virion measures 100 to 150 nm in diameter as observed in electron microscopy. Mature viral particles are characterized by an electron-dense conical core. The core is surrounded by a lipid envelope, which is acquired as the virion buds from the infected cell. The virion core contains two copies of single-stranded positive-sense genomic RNA, each of which encodes the complete viral repertoire of structural, enzymatic, and regulatory proteins. The HIV genome is approximately 10kb in length and is organized similarly to that of other retroviruses. The sequence is flanked by two long-terminal repeats (LTRs). The 5' LTR contains the enhancer-promoter sequences for viral transcription, and the 3' LTR contains the polyadenylation signal. From 5' to 3' the viral genome contains the gag gene, which encodes the virion structural components; the pol gene, which encodes the viral enzymes; and the env gene, which encodes the envelope glycoproteins. The primate immunodeficiency viral genomes contain six genes in addition to gag, pol, and env (Emmerman et al., 1998). These are vif, vpr, tat, rev, nef, and either vpx (in HIV-2 and SIVsm) or vpu (in HIV-1 and SIVcpz). The products of these additional genes function via interactions with host cell proteins to optimize viral replication by a variety of mechanisms discussed below (Strebel et al., 1999).

2.2.3.3.2 Major Structural and Regulatory Proteins

The major structural and core proteins of HIV are synthesized from gag as a large, myristoylated precursor protein (pr55), which is subsequently cleaved by the viral protease to yield the matrix (MA) (p17), capsid (CA) (p24), and nucleocapsid (NC) (p7) proteins. The matrix protein is primarily a peripheral membrane protein located along the inner leaflet of the viral lipid envelope, where it
directs the incorporation of the envelope glycoproteins (Env) into the forming virion (Dorfman et al., 1994). Some p17 is also found in the virion core, where it participates in the transport of the viral preintegration complex to the nucleus (Bukrinsky et al., 1993). The capsid protein p24 assembles to form the conical core of the virion. The core structure has been proposed to follow the principles of a fullerene cone composed primarily of a curved array of hexameric CA subunits, with the inclusion of several pentameric subunits to allow closure of the cone (Li et al., 2000). The nucleocapsid protein (p7) is an RNA binding protein required for packaging of the genomic RNA into the virion. Several smaller cleavage products- p1, p2, and p6, are also generated from the p55 precursor. The p6 protein contains a so-called late or L domain, which is required for viral budding, and it mediates the incorporation of the viral accessory protein Vpr into virions (Accola et al., 2000).

The viral enzymes are also produced by proteolytic cleavage from a large precursor molecule. During translation, a ribosomal frameshift occasionally occurs between the gag and pol open reading frames, resulting in the synthesis of a gag-pol precursor protein (Pr170) (Jacks et al., 1988). The ratio of Gag-Pol to Gag produced is approximately 1:20. Subsequent cleavage of pr170 yields the Gag protein products and the retroviral enzymes; protease (p11), reverse transcriptase (RT)/RNase H (p66/p51) and integrase (p32). The aspartyl protease cleaves the structural and enzymatic proteins from the large polyprotein precursors. This enzyme is a symmetric dimer, and it is activated by dimerization during virion assembly (Kaplan et al., 1994). RT is a heterodimer with 66 and 51 kDa subunits. This enzyme provides both the RT activity, which allows RNA-dependent DNA polymerization, and RNase H activity, which allow the specific degradation of RNA present in RNA-DNA hybrid duplexes. The RNase H domain is present in the 66 kDa subunit (Davies et al., 1991). The RT activity also resides in the p66 subunit,
with the p51 subunit serving as a scaffold. Integrase mediates insertion of the viral DNA into that of the host cell (Farnet et al., 1996). To accomplish this enzyme utilizes two activities: a DNA-cleaving activity, which cuts the host DNA and processes the ends of the HIV-DNA, and a DNA-joining activity, which covalently attaches the viral DNA to that of the host via strand transfer reactions.

The protein product of the env gene is synthesized in the endoplasmic reticulum as a 88-kDa polypeptide (Willey et al., 1988). This protein undergoes glycosylation in the endoplasmic reticulum and golgi network. The resulting molecule, gp 160, contains N-linked, high-mannose sugars, which account for approximately half of the final molecular mass of 160 kDa. Much of the newly synthesized gp 160 is retained in intracellular compartments and eventually degraded; however, a fraction is cleaved by the cellular serine protease, furin, to generate the transmembrane (gp41) and surface (gp120) subunits (Willey et al., 1988).

The primate immunodeficiency viruses encode a number of nonenzymatic and nonstructural gene products (Emerman et al., 1998). Two of these genes, tat and rev, are essential for viral replication. The others (vif, vpr, vpx, vpu and nef) are dispensable for viral replication under certain conditions in vitro and consequently have been termed accessory genes. The 14-kDa trans-activating protein-Tat, markedly enhances the rate of transcription from the 5' LTR by recruiting cellular factors that enhance the processivity of the cellular RNA polymerase II complex. Tat recruits a cyclin-dependent protein kinase complex (Cdk9 plus cyclin T) to a structured region near the 5' end of the primary viral transcript (the Tat activation region) (Wei et al., 1998). Cdk9 then hyperphosphorylates the C-terminal domain of RNA polymerase II, which allows
efficient elongation of the nascent viral mRNA (Okamoto et al., 1996) In the absence of Tat, viral transcription is essentially stalled just after initiation.

The 18-kDa Rev protein mediates the transport to the cytoplasm of singly spliced and unspliced viral RNAs; these encode the HIV-1 structural and enzymatic proteins and include the viral genomic RNA. Rev directly links these viral RNAs to a specific nuclear export pathway. In the absence of Rev activity, viral mRNAs are inefficiently exported from the nucleus, and they undergo extensive splicing to yield subgenomic mRNAs encoding only a subset of viral proteins. The 27 kDa Nef protein enhances viral replication and pathogenicity by modulating both cellular signal transduction and membrane trafficking.

The 14 kDa HIV-1 Vpr protein appears to have two distinct functions. First, expression of Vpr arrests cells in the G2 phase of the cell cycle. The reasons for this cell cycle arrest are unclear, but enhanced viral transcription during the prolonged G2 phase appears to increase the yield of progeny virions per replication cycle (Goh et al., 1998). Secondly, Vpr has an affinity for the cell nucleus and is a component of the viral preintegration complex, which forms after viral entry and uncoating and which contains the viral cDNA and integrase (Miller et al., 1997). The 23 kDa Vif proteins are required for the production of infectious virions. The basis for the effect of Vif on infectivity is not clear but may relate to enhanced stability of the viral core and preintegration complex (Simon et al., 1996). This stability appears to allow more efficient synthesis of viral DNA in newly infected cells. The 16 kDa Vpu proteins is an integral membrane protein encoded by HIV-1
but not by HIV-2 or SIV. Vpu enhances the release of virions from infected cells (Klimkait et al., 1990).

2.2.3.4 Epidemiology

2.2.3.4.1 Distribution and Geography

The AIDS pandemic can be viewed as a composite multiple epidemics, each occurring in specific geographic regions and populations. While HIV-2 has remained confined largely to West Africa and European immigrants, HIV-1 has spread throughout the world. Group M viruses are responsible for the vast majority of HIV-1 infections. Viruses belonging to the distinct subtypes or clades within group M have been isolated in geographically distinct regions of the world. Subtype C is currently predominant in the global epidemic. In the United States, Europe and Australia, subtype B predominates illustrating a founder effect in which one or several viral variants were introduced and then disseminated through the population (Foley et al., 2000). Subtype B is found rarely in Africa. Instead, subtype A predominates in West Africa, subtypes A and D predominate in East Africa, and subtype C predominates in Southern Africa. Variants of HIV-1 appear to quickly expand to become the major subtype when introduced into a specific population or geographic area. For example, in Thailand, subtype B viruses predominated among intravenous drug users in Bangkok, while CRF-01AE (formerly designated subtype E) recombinant viruses spread throughout the country by heterosexual contact. No evidence that subtypes, display differences in biology or pathogenesis has yet been generated.

The distribution of HIV-1 subtypes is complex and probably reflects a stochastic dissemination. Multiple subtypes co-circulates within areas of central Africa, Southeast Asia, and South America. According to world health organisation
estimates, over 22 million people were estimated to have died since the beginning of the pandemic and over 36 million were living with HIV-infection or AIDS in 2001 (Piot et al., 2001). The incidence of infection on a worldwide scale is estimated to be 16,000 per day. 5.3 million new cases were estimated to have occurred during the year 2000. Approximately 95% of all HIV infections have occurred in young and middle-aged adults in the developing world. The prevalence and incidence rates of HIV infections vary widely throughout the world and reflect the progress of local epidemics, fueled by distinct modes of transmission, socioeconomic environments, and behavioral factors (De Cock et al., 2000 and Piot et al., 2001). Sub-Saharan Africa accounts for over two-thirds of the current cases of HIV infection, although it contains only 10% of the world’s total population.

Asia accounts for almost 20% of worldwide cases of HIV-1 infection, with 5.8 million cases as of 2000. The majority of these, approximately 4 million, have occurred in India. However, Thailand is the Asian country with the highest adult prevalence, approximately 2%. During 2000, 7,80,000 new cases were estimated to have occurred in South and Southeast Asia. Rapid increases in the spread of HIV infection are occurring in Cambodia, Malaysia, Vietnam, and China. Latin America accounts for 4% of worldwide cases of HIV-infection, with 1.3 million cases. In Latin America, 1,50,000 new cases were estimated during 2000. The Caribbean accounts for 1% of worldwide cases, with approximately 3,90,000 total cases and an adult prevalence of 2.3%. Eastern Europe and Central Asia now account for 7,00,000 cases. In this region, 130,000 new cases were estimated in 1999, but 2,50,000 new cases were estimated in 2000.
2.3 HIV AND HBV

HBV is the leading cause of chronic liver disease worldwide, including hepatitis, cirrhosis, and hepatocellular carcinoma (Mahoney et al., 1999). Given the epidemiological similarities of Hepatitis B virus (HBV) and HIV infections, it is not surprising that markers of past HBV infections namely hepatitis B surface antibody (anti HBs) or hepatitis B core antibody (anti HBe), are found in approximately 90% of AIDS patients (Rogers et al., 1983; Rustgi et al., 1984 and Lebovics et al., 1985). The prevalence of chronic HBV carriers, as determined by hepatitis B surface antigen (HBsAg) positively, among AIDS patients with evidence of past HBV infection is about 10% (Rustgi et al., 1984; Rogers et al., 1983 and Lebovics et al., 1985). Until recently, clinical manifestations of chronic hepatitis B in persons infected with HIV were uncommon because of competing risk for mortality from opportunistic infections associated with immunosuppression. Over the past several years, effective antiretroviral medications have increased the life expectancy of HIV-infected persons, resulting in a changing spectrum of HIV-associated diseases. Chronic liver disease from hepatitis C virus (HCV) has been recognised as a HIV associated opportunistic infection and hepatitis B is being included. Of additional concern is the rising incidents of acute hepatitis B in high-risk groups because of poor compliance with hepatitis B vaccinations; thus HIV/HBV coinfection may become a growing problem.

2.3.1 HIV/HBV interaction

Active HBV infection in HIV patients may lead to direct and indirect viral interactions. To interact directly would require both viruses to be present in the same cell. HBV primarily affects hepatocytes, but has been found within the lymphoid system. In one study HBV-DNA was detected in peripheral blood
mononuclear cells (PBMC) of all patients coinfected with HIV and HBV but only 50% of PBMC of patients infected with HBV alone (Noonan et al., 1986). Likewise HIV has been found within the liver, not only the Kuppfer (Schmitt et al., 1990) and sinusoidal liver cells that express CD4 markers, but also hepatocytes which lack CD4 receptors (Cao et al., 1990).

2.3.1.1 Molecular Interactions

2.3.1.1.1 Direct effect of HBV on HIV replication

On entering cells, HIV-RNA is transcribed to proviral DNA flanked by long terminal repeat sequences which have enhancer and promoter regions which can be activated by cytokines and transactivating factors to induce HIV replication. The molecular mechanism by which cytokines affect HIV replication is best understood for TNF-alpha which activates the nuclear transcription factor NF-kB to induce expression of numerous cellular genes. In unstimulated cells, NF-kB is found in the cytoplasm bound to its inhibitor I-kB, but when cleaved from its inhibitor protein, NF-kB enters the cell nucleus and up-regulates HIV replication. The hepatitis B transactivator, HBX protein, is coded by the HBX gene and appears capable of up regulating, not only HBV gene expression but also expression of reported genes under the control of HIV (LTR) sequences by using NF-kB (Seto et al., 1988 and Twu et al., 1989).

A further analysis of the HIV LTR revealed that the region from bases \(-105\) to \(-80\), which contains the two NF-kB binding domains, is responsible for the transactivation by HBX proteins. Since the HBV X protein does not bind directly to DNA, it presumably interacts with NF-kB or kB- like transcription factors within the cell to activate cellular genes involved in HBV replication, and it is possible that a
similar mechanism operates in vivo to enhance HIV replication in cells containing both HIV and HBV.

Clearance of HBV virus and subsequent liver damage is mainly mediated by cytotoxic T cells. Therefore a reduction in number and function of T cells, as seen in HIV disease, will have an influence on HBV infection. Studies in patients with chronic HBV infection and HIV associated immunosuppression have shown a reduction in hepatic transaminases and necroinflammation, despite a higher HBV DNA load (Perrillo et al., 1986; Krogsgaard et al., 1987; Bodsworth et al., 1989 and Goldin et al., 1990) However, a recovery in the number and function of T cells following effective anti-HIV therapy will have the converse effect.

2.3.1.1.2 Indirect effects of hepatitis B virus on Human Immunodeficiency Virus Replications

HIV replication is known to be affected by a number of cytokines (McNair et al., 1991). TNF has been shown to upregulate HIV expression from chronically infected monocytes (Poli et al., 1990) and probably does so by inducing NF-kB. Interferon alpha however, has an antiviral action. It does not appear that HBV is capable of increasing HIV replication by inducing the secretion of specific monokines. Although some viruses of the herpes group have been shown to augment HIV expression by inducing monokine production, similar studies on HBV have failed to demonstrate any effect (Clouse et al., 1989).

The importance of endogenous interferons in controlling HIV is not known. In vitro, interferon alpha decreases the expression of HIV in infected T cells and monocytes, (Kornbluth et al., 1989) and its likely that it plays a role in controlling HIV expression in the early stages of infection. Recent evidence suggests that the HBV pol gene product inhibits the cellular response to interferons and may
also decrease the production of interferon in response to viral infection (Foster et al., 1991). For example, the response to interferon was inhibited when cells were transfected with the plasmid designed to express the pol gene, along with and interferon inducible reporter construct. Further analysis revealed that the region responsible was the HBV terminal protein. A cell line was subsequently derived that stably expressed the HBV terminal protein; these cells did not respond to interferon alpha or gamma, nor did they produce interferon-B when exposed to double stranded DNA. Therefore, the presence of HBV in HIV infected mononuclear cells may abrogate the antiviral effect of endogenous and therapeutic interferons on HIV replication and in doing so may shorten the duration latency.

Other possible ways in which HBV may interact with HIV at the mononuclear or immunologic level more speculative. HBV replicates via RNA intermediate, which is reverse transcribed, probably by the HBV polymerase, back to DNA. It is conceivable that in lymphocytes infected with both HIV and HBV the reverse transcriptase activities of each virus could interact to enhance the formation of proviral or genomic DNA, respectively. However no evidence exist that this occurs in vitro or in vivo and the requirement of the two enzymes for different types of primer makes it unlikely (McNair et al., 1992).

2.3.1.2 Effects of HIV on Hepatitis B virus

HIV infection of CD4 positive lymphocytes and macrophages leads to a progressive decline in cell mediated immunity. This is partly mediated by destruction of the infected and probably uninfected T cells and partly by indirect effect on antigen presentation and the cytokine network. Cell-mediated immunity plays a central role in the pathogenesis of HBV. Cytotoxic T lymphocytes are thought to be responsible for the clearance HBV infected hepatocytes (Kakumu
et al., 1980 and Naumov et al., 1984) when immune recognition occurs in acute HBV infection or in chronic disease with the spontaneous or interferon induced viral clearance. Factors that impair cell-mediated immunity are therefore likely to modify course of HBV infection by allowing uncontrolled viral replication and reducing hepatocellular damage. This has been recognized previously in non-HIV infected patients receiving immunosuppressive drug therapy (Hoofnagle et al., 1982). Since HIV also primarily affects cell-mediated immunity, it would be expected to influence HBV infection. The elimination of HBV infected hepatocytes is dependent on an intact cellular immune response and the presentation of HBV-related peptides in association with MHC class I antigens on the cell surface. MHC class I display is enhanced by interferon alpha and gamma, and it is thought that this action of interferon is important for the successful therapy of chronic HBV. Mononuclear cells from AIDS patients generate diminished levels of interferons alpha and this may impair antigen presentation, allowing increased replication of HBV.

2.3.1.3 Clinical course of Human Immunodeficiency and Hepatitis B viral coinfection

In most patients with HIV and HBV coinfection it is impossible to determine the relative timing of the two viral exposures. Fortuitously from some HBV vaccine trials retrospective can be done. The major epidemic of HBV in homosexual men predated the outbreak of HIV. There is epidemiologic evidence that individuals with low helper to suppressor T cell ratios are more susceptible to HIV infection (Ludlam et al., 1985). One study of hepatitis B surface antigen (HBsAg) carriers without AIDS related symptomatology noted an elevated level of T suppressor and cytotoxic cells and functionally deficient T helper cells (Yoffe et al., 1984). It is possible that by modulating the immune system in this way HBV may predispose to infection with HIV. However, it can also be argued that the
minority of adults who develop chronic viral carriage of HBV following viral exposure have already demonstrated an immunodeficiency and it has been noted in a retrospective study that this group of patients may have a decreased ability to produce interferon (Ikeda et al., 1986). This effect may however be secondary to HBV infection.

\[2.3.1.4 \text{ Epidemiology of HIV and HBV coinfection}\]

Approximately 400 million persons worldwide have chronic hepatitis B, which is defined as the presence of HBsAg for a minimum of 6 months, and between 5,00,000 and 1 million persons die annually of HBV related disease (Mahoney et al., 1999). In the United States, there are approximately 335000 incident cases annually (Coleman et al., 1998) and about 1 million person chronically infected. Coinfection with HBV and HIV is common, with 70 to 90% of HIV infected individuals having evidence of past or active infection with HBV (Gilson et al., 1997 and Rodriguez-Mendez et al., 2000). The prevalence of HBsAg chronic carriage among HIV infected individuals is 1.9 to 9% (Saillour et al., 1996). Among IVDUs, 90% of HIV infected individuals have evidence of exposure to hepatitis B (hepatitis B core antibody (anti-HBc positivity) and 60% also have evidence of fast infection with presence of hepatitis B surface antibody (anti-HBs) (Rodriguez-Mendez et al., 2000). Additionally, anti-HBc is more frequently found in HIV infected homosexual men compared to those who are HIV seronegative (72% vs. 31% respectively).

\[2.3.2 \text{ Influence of HBV on the course of HIV disease}\]

There are conflicting data with respect to the impact of HBV on the course of HIV infection. While some studies have shown an increased rate of HIV progression to AIDS among individuals with markers of exposure to HBV (anti
HBC) (Eskalid et al., 1992), others have not shown any change in the progression of HIV disease or survival (Gilson et al., 1997). In the largest prospective cohort of 3,040 HIV seronegative homosexual men, 296 underwent HIV seroconversion during the 4 year follow-up period. The likelihood of HIV infection was associated with serologic markers of HBV (RR 2.03 for HBV carrier and 2.22 for HBV immune), an association that remained ever after adjustment for sexual behaviour or sexually transmitted disease over time (Twu et al., 1993). However there was no significant increase in the rate of progression to AIDS in these individuals over a 2.5 year follow up (Solomon et al., 1990). Therefore HBV does not appear to significantly influence progression of HIV infection but may correlate with risk for HIV infection in some population.

2.3.3 Influence of HIV on the course of HBV infection

The course of acute hepatitis B may be modified in the presence of HIV with lower incidence of icteric illness and a higher HBV carriage rate of about 25% compared to about 5% in those uninfected with HIV (Horvath et al., 1994 and Gatanaga et al., 2000). In chronic infection, markers of HBV replication appear to be influenced by HIV infection. There is a trend towards lower rate of clearance of HBeAg and HBV DNA viral load (Weller et al., 1986 and Perrillo et al., 1986). Additionally, HIV induced immunosuppression may result in lower serum transaminases possibly due to a reduction in the severity of liver disease (Kroggaard et al., 1987). However, immunosuppression may also be associated with reactivation of HBV infection in persons who have lost detectable HBsAg or HBeAg (Waite et al., 1988 and Mastroianni et al., 1998). Although in HIV infected individuals, symptomatic reactivation and loss of anti-HBs is uncommon (Rodriguez-Mendez et al., 2000 and Horvath et al., 1994), asymptomatic reactivation or reinfection occurs frequently in patients who develop AIDS, leading
to a significantly higher prevalence of HBsAg (Horvath et al., 1994). A prospective cohort study of 152 untreated homosexual male HBV carriers and 212 HBsAg-negative controls, of whom 41% and 70% respectively, were seropositive for HIV, assessed the effect of HIV on the natural history of HBV (Gilson et al., 1997). After a mean follow-up of 2.8 years, serum HBV-DNA levels were higher, ALT levels were lower and loss of serum HBsAg occurred at a lower rate in HIV carriers compared with HIV uninfected carriers (relative hazard, 0.39, CI 0.16-0.94) (Gilson et al., 1997). Therefore, HIV seropositivity has been associated with significantly lower ALT levels, higher serum HBV-DNA levels, lower rate of HBeAg and serum DNA clearance, decreased liver injury, and an increased loss of antiHBs.

In 1975 Szmuness et al. reported the implication of sexual behavior in transmitting HBV. They have found that persons who had higher –than –average probability of exposure to potentially infective partners were found to have serologic evidence of hepatitis-B and also found out the involvement of patterns of sexual behavior like anal intercourse in the transmission of hepatitis B.

Lebovics et al. (1985) reported the serologic evidence of hepatitis B virus in HIV infected patients. Histological evidence was observed in two HBsAg positive patients despite abnormal liver chemistries, and the lack of inflammation in limited HBsAg carriers was attributed to a marked depression in T helper cell activity, but high rate of hepatitis B seroconversion was contradictory to this (Gorden et al., 1986). Elimination of virus particle in immunodeficient state is not possible, so it was postulated that seroconversion might have taken place before the onset of acquired immunodeficiency syndrome.

Hepatic inflammatory response in HBV and HIV positive cases was reported by number of authors. Hepatic inflammation was less severe and
HBV-DNA replication was more in HIV positive homosexual men rather than HIV negative homosexuals and heterosexuals with chronic hepatitis B infection (Perrillo et al., 1986). The serum AST level was low while HBV-DNA level was high in male homosexuals with chronic HBV infection with HIV (Krogsgaard et al., 1987). On the contrary, serum HBV DNA and AST concentration were unrelated in homosexual males with chronic Hepatitis B and antibody to HIV and the presence of other factors which may modify the hepatic inflammatory responses in chronic HBV infections as there was no relationship found between cellular immune function and HBV replication (Rector et al., 1988).

HIV superinfection in an HBsAg/HBeAg positive male homosexual with chronic hepatitis caused a rapid fall of ALT activity and HBV replication was very high (Pastore et al., 1988). This was implicated with the impaired T-cell function during acute HIV infection. McDonald et al., 1987 clearly shown that male homosexual HBV carrier positive for anti-HIV may be immunosuppressed before there are clinical signs of immunodeficiency, and this allows an increased level of replication of hepatitis B virus.. They also reported the less percentage (%) of HBV-DNA in anti-HTLV III positive group in comparison to the anti HTLV-III negative group, and also found homosexual men with HBeAg positive chronic liver disease who are anti-HTLV-III positive were less responsive to the direct anti-viral and immunomodulatory effects of recombinant IFNα 2a. Waite et al. (1988) reported the involvement of HIV in the reappearance of HBsAg.

The healthy anti-HIV carriers are no more likely to be HBsAg carriers than HIV seronegative subjects. However, immunodeficiency induced previously by HIV infection is likely to be responsible for the high prevalence of HBsAg among AIDS patients.
Bodsworth et al. (1989) studied the effect of concurrent HIV on chronic Hepatitis B of male homosexual patients with antibodies to HIV were more likely to express Hepatitis B e antigen. In HBeAg seropositive subjects, concurrent HIV infection was associated with lower serum ALT level. They concluded that HIV infection over chronic hepatitis B may reduce the severity of HBV but viral replication may make it more contagious and resistant to antiviral therapy.

In 1990, Halder et al., reported that the effect of HBV infection in homosexual men with prior HIV infection. Persons with HIV-1 infection and HBV vaccinated preceding HBV infection had a significantly higher risk of developing HBV carriage, viremia, prolonged ALT elevation and clinical illness. HBV carriage was increased in unvaccinated and those who failed to respond to vaccination among HIV-1 infected men. Risk of HBV carriage also increased in persons who received vaccine at the time they developed new HBV infection suggesting inactivated hepatitis B vaccine may temporarily impair the immune response to HBV infection in HIV-1 infected persons.

According to Koblin et al. (1992), HIV had no effect on HBV replication, because viral replication was similar among patients negative for antibody to HIV type-1. DNA level of hepatitis virus did not increase with the decline of cellular immunity. They have concluded that HBV replication might have been a function of duration of HBV infection rather than the effect of HIV type-1 among chronic carriers, that too, it was inversely proportional to HBV-DNA and HBeAg positivity. But other reports revealed that HBsAg carriers with HIV-1 infection have higher levels of HBV replication (Perrillo et al., 1986; Krogsgaard et al., 1987; Novick et al., 1988; Govindarajan et al., 1988 and Bodsworth et al., 1989).
Bodsworth et al. (1991) reported the role of HIV-1 infection in developing chronic HBV infection. They have noticed the conversion of acute viral hepatitis to chronic HBV infection in HIV-1 infected homosexual men. The HBV-DNA positivity and severe liver damage occurs in both HIV positive and HIV negative chronic HBV carriers but the clearance rate of HBV-DNA was lower in the HIV positive subjects than in the HIV-negative subjects.

The hepatitis B virus markers were more prevalent in patients with AIDS compared with patients without AIDS. Anti-HBs, which typically reflects immunity, did not differ between patients with or without AIDS, where as HBsAg, which indicates chronic infection, was more than twice as prevalent in the AIDS group (Scharschmidt et al., 1992). Finally they suggested that patients with the more advanced immunosuppression characteristic of AIDS may be less likely to clear HBV infection after exposure or more likely to reactivate latent HBV infection or both.

Several reports (Taylar et al., 1988 and Bodsworth et al., 1991) published the role of HIV infection in exposing HBV. Other reports concluded that HIV interferes with HBV life cycle by extending the period of active replication (Krogsgward et al., 1987 and Bodsworth et al., 1991) or by reactivating HBV replication without an increase in inflammatory liver activity (Krogsgward et al., 1987 and Bodsworth et al., 1991) but Oscar Kasha et al. (1994) stated that prevalence of serum HBsAg does not correlate with sero positivity, which in turn revealed that HIV facilitate the persistence of HBV carrier state however additional evidence of the same study reported HBsAg seroprevalence increased with the transition from asymptomatic to AIDS. To summarize, HBV reactivation may occur more often in patients with AIDS rather than asymptomatic. HBV replication is
more prevalent in AIDS patients, and the HBV-DNA replication is frequent in patients with low CD4 cell counts (Bodsworth et al., 1991).

The difference in mean ALT measurements was not observed in HIV antibody positive patients when compared with the HIV antibody negative patients but the prevalence of HBeAg was significantly higher in HIV antibody positive group than HIV antibody negative group (Ann et al., 1996). The same results was observed by Bodsworth et al., 1989; Mc Donold et al., 1987 and Goldin et al., 1990 but some other reports declared that there was no difference in the prevalence of HBeAg according to HIV antibody status (Bodsworth et al., 1989; Sunen et al., 1992 and Koblin et al., 1992). They have concluded that the mortality rate due to AIDS was very high when compared with the death rate due to liver failure, hence, the main target for therapy should be HIV rather than HBV.

Coinfection with HIV and hepatotropic viruses causes complex interactions. HIV induced impairment of the cell-mediated immunity causes higher replication of hepatotropic viruses (Twu et al., 1989 and Housset et al., 1992). Additionally HBV leads to enhanced transcription of HIV through an NF-KB element in the long terminal repeat of HIV (Twu et al., 1989). HCV also activates HIV replication through cytokines like tumor necrosis factor-alpha (Horvath J et al., 1994). However the impact of these coinfection on the clinical out come of HIV infected patients is unclear. Most of the reports suggest that the infection of HIV with either HCV (Martin et al., 1989 and Soriano et al., 1995) or HBV (Eskild et al., 1992) accelerates the clinical course of HIV-infected patients. In contrast, other studies have not revealed significant progression of the disease in HIV infected patients with either hepatitis B (Halder et al., 1991) or hepatitis C (Wright et al., 1994) coinfection.
Johann Ockenga et al. (1997) published that the coinfection with hepatitis B and hepatitis C is common and in case of prolonged survival of HIV infected patients, HBV or HCV infection may become an important clinical problem. Coinfection with HBV or HCV was associated with a reduced survival.

The enhanced level of HBV replication associated with HIV infection demonstrated in many of the cross sectional studies are accompanied by a significantly lower rate of spontaneous reduction in viral replication with time. A significant reduction in the annual rate of loss of serum HBV DNA detectable by dot-blot hybridization assay, from 20% in HIV uninfected to 4% in HIV infected patients, but no effect on HBeAg to HbeAb seroconversion has been reported (Krogsgaard et al., 1987).

Earlier a report revealed that rate of loss of serum HBeAg was reduced from 11.5% to 4.8 % per annum, although the difference was not statistically significant (Weller et al., 1986). Gilson et al. (1997) revealed that the prolongation of a period of high rate of viral replication in HBV carrier who are also HIV infected. HIV may both increase and prolong the infectivity of HBV. This may have consequences for the pool of HBV carrier and their infectivity especially the areas of the world where HBV prevalence is high, which are also the region with the major burden of HIV disease-Africa and Asia, so that the increased infectivity could have a major effect on the spread of HBV infection both horizontally and vertically.

HIV infection may reduce the activity of HBV-induced inflammatory liver disease. However it is not known how this may translate in to the risk of development of hepatocellular carcinoma. However the investigation could only be undertaken if there are a larger number of long-term survivors with HIV infection (Gilson et al., 1997).
Colin et al. (1999) assessed the liver function biochemical tests, serum HBV DNA, and histology activity in a series of 132 (65 anti-HIV positive) homosexual non-drug addicted men with chronic hepatitis B. In that study all the HIV positive patients had lower serum alanine transferase activity (p=0.0001), lower serum albumin (p=0.0009) and higher HBV-DNA levels than HIV seronegative patients, and they also observed higher prevalence of cirrhosis in anti-HIV positive patients without increased liver necrotico-inflammatory process.

Mutation associated with hepatitis B virus (HBV) resistance to lamivudine have not been extensively addressed in HIV-HBV coinfection. In one study the HBV polymerase sequence from nine coinfected patients who experienced HBV recurrence while under lamivudine treatment was observed (Thibault et al., 1999). In HIV-HBV coinfected patients, treatment with high daily doses of lamivudine does not prevent the emergence of HBV resistant strains carrying mutations with in the polymerase B and C domains, which also confer resistance to other nucleotide analogues (Lander et al., 1998).

In HIV-HBV coinfected patients, inhibition of HBV replication had been obtained in less than 8% of the cases with interferon alfa (McDonald et al., 1987 and Wong et al., 1995). Lamivudine, an oral nucleotide analogue is effective against both HIV and HBV replication. Lamivudine has promptly inhibited HBV replication in more than 80% of cases in both HIV and non-HIV-infected patients (Benhamou et al., 1996 and Lai et al., 1998). However, HBV resistance to lamivudine caused by HBV-DNA polymerase gene mutations has been reported in both liver transplanted and immunocompetant patients (Niesters et al., 1998). Incidence of HBV resistance to lamivudine is of 14% to 27% after 1 year treatment in non-HIV infected patients (Dienstag et al., 1995; Benhamou et al., 1996 and Lai et al., 1998).
A high incidence of hepatitis B virus (HBV) resistance to lamivudine in human immunodeficiency virus (HIV)-infected patients was reported, close to 100% at 5 years, underlying the need for alternative therapies (Benhamou et al., 1999). Indeed, although HIV-related immunodepression may reduce liver necroinflammatory lesion and serum alanine transaminase (ALT) level in HBV-infected patients (Perrillo et al., 1986). HBV infection has been shown to be associated with more severe liver fibrosis in HIV-coinfected patients, (Colin et al., 1999) which may increase mortality.

The increasing complexity of HIV treatment regimens and high prevalence of co-infection with HBV and HCV made demand to ransack the accurate information regarding the risk of hepatotoxicity associated with antiretroviral drugs to guide appropriate use of these drugs. In the view of this hepatotoxicity in association with different types of retroviral drugs and the role of hepatitis C or B virus infection, Sulkowski et al. (2000) observed severe hepatotoxicity in 31 (10.4%) of 298 patients, and they found that Retinovir was associated with a higher incidence of toxicity (30%). However, there was no significant difference in hepatotoxicity of other treatment groups like NRI, Nelfinavir, Saquinavir and Indinavir. Hepatitis virus B or C infection also caused severe hepatotoxicity but in patients not taking retinovir. Similar results was also observed by Rodriguez rosedo et al., 1998 but with the use of HAART. Sulkowski et al. (2000) concluded that CD4 cell recovery during ART was associated with severe hepatotoxicity. However this finding may reflect medication adherence rather than immune mediated liver injury.

The active ART created the possibility of severe hepatotoxicity during HBV and HCV combination in HIV-1 positive cases (Brinker et al., 2000). HIV-1 infected patients co infected with HBV or HCV were at considerably higher risk of
developing LEE (Liver Enzyme Elevation) when HAART initiated, but modification of ART was not suggested. Several mechanism were postulated to play a role in the increased risk for LEE (Kakumnu et al., 1980; Naumov et al., 1984; Perrillo et al., 1986 and Powderly et al., 1998) but Brinker et al., 2000 could not find a clear relationship of seroconversion with LEE.

In one study 14% of lamivudine resistant HBV in Lamivudine treated HIV-1/HBV coinfected patients and also found that the resistance in both HBV & HCV developed by parallel evolution (Deenan Pillly et al., 2000). This report urged the requirement for additional potent anti-HBV agents which, when used in combination, could inhibit replication to a greater degree to that afforded by monotherapy and consideration should be given to particular drug combination with potentially non-overlapping resistant patterns (Ono-nita et al., 1999).

The heterosexual exposure resulted in higher percentage of hepatitis B virus coinfection, all except one of the patients they have analyzed acquired infection through heterosexual exposure. In addition to that males were more likely to be coinfected than females, it was statistically significant (Sud et al., 2001). The more prevalence of transmission of HBV by heterosexual contact, in a racially mixed community in Florida was published by Rosen blum et al., 1990 and Castro et al., 1988. These reports are contradictory with other reports which highlights the possibility of coinfection by homosexual contact (Ann et al., 1996 and Richard et al., 1997).

In HIV infected persons, clearence rates on HBsAg and HBeAg are decreased compared to those without HIV infection (Gilson et al., 1997) and may be related to the degree of immunosuppression (Bodsworth et al., 1991). In a prospectively followed cohort, HIV infected men had HBeAg loss of 12% in 5 years
compared to 49% in the HIV-seronegative men (Gilson et al., 1997). HIV infection is also associated with reactivation of HBV (Vento et al., 1989) accelerated loss of anti-HBs (Biggar et al., 1987) higher levels of HBV DNA, (Colin et al., 1999 and Gilson et al., 1997) and lower ALT levels (Colin et al., 1999). The lower ALT levels suggest less hepatocyte destruction because of a depressed immune response and may contribute to the decreased effectiveness of anti-HBV therapy incoinfected individuals.

A severe, rapidly progressive form of liver disease described in liver transplant recipients, fibrosing cholestatic hepatitis, leads to liver graft rejection and is characterised by the ballooning degeneration of hepatocytes and high HBV antigen expression in the liver (Davies et al., 1991). This rare disease is thought to be due to a direct cytopathic effect of the virus and has also been described in patients with HIV infection (Fang et al., 1993).

### 2.3.4 HIV-HBV co-infection and highly active antiretroviral therapy

Flares of liver enzymes in HIV-HBV coinfected patients receiving highly active antiretroviral therapy (HAART) can arise from one or more of the following causes, so a careful evaluation is required to discern the etiology prior to discontinuing or changing an effective HAART regimen. First, HBV infection clearly increases the risk for toxicity from antiretroviral medication (Saves et al., 1999 and Sulkowski et al., 2000); however, only a few experience a severe, reversible hepatotoxicity (ALT >5 X the upper limit of normal (ULN) usually with in the first 6 months of starting a regimen. Coinfected patients are also at increased risk for the rare condition of hepatic steatosis and lactic acidosis from the nucleoside analogues, which can occur after years of therapy. Second, a flare may herald HBeAg seroconversion, so it is not unreasonable to continue HAART if this is a
Third, immune reconstitution has been reported to lead to ALT elevations in patients with chronic hepatitis B (Velasco et al., 1999). Fourth, there have also been several case reports of reactivation or exacerbations of HBV after discontinuing lamivudine as part of a HAART regimen (Altfeld et al., 1998 and Bessesen et al., 1999), and it is well described in the development of HBV resistance to lamivudine (Bessesen et al., 1999). Fifth, reactivation of HBV replication has also been described in the setting of HAART independent of lamivudine withdrawal or resistance (Manegold et al., 2001). Last, superinfection with another hepatotropic virus must be considered.

2.3.5 Prevention of HBV infection

Vaccination of persons at risk for acute HBV infection is the most effective means of prevention. After the standard vaccine dose, individuals with titres below 10 IU/L should be considered for revaccination (CDC 2002). HIV-HBV coinfected adults manifest decreased response to the hepatitis B vaccine. Their seroconversion rates to the inactivated plasma derived and the recombinant three doses HBV vaccine are 50 to 56% (Collier et al., 1988) and 20 to 30, (Bruguera et al., 1992 and Keet et al., 1992) respectively, compared with the general population rate of 90%. This decreased antibody production rate appears to be independent of the mode of acquisition of HIV and tends to be more prominent with lower CD4 counts (Keet et al., 1992). A fourth immunization has had limited success in improving these responses (Keet et al., 1992). Rey et al demonstrated that three additional doses separated by 1 month each increased the response rate in coinfected individuals to 90%; however, after 1 year only 59% of them maintained protective anti-HBs titers (Rey et al., 2000). If response to the vaccine does not occur, then other prevention methods, including barrier precaution to prevent sexual
transmission; substance abuse counseling and avoidance of tattooing, body piercing, and sharing personal care items, should be discussed.

The Centers for Disease Control & Prevention (CDCP) recommends annual anti-HBs titers in hemodialysis patients with booster doses when levels fall below 10IU/L because their immunologic memory may be less complete. Data do not exist regarding routine monitoring of anti-HBs titers and booster vaccinations in HIV-infected patients. However, it seems prudent to consider annual monitoring as has been recommended by the European Consensus Group on Hepatitis B immunity and to consider booster doses when the titer falls below 10 IU/L, especially in patients who have responded to HAART.

2.3.6 Treatment of chronic hepatitis B in HIV-infected persons

The goal of chronic hepatitis B treatment is to prevent the development of cirrhosis rather than viral eradication because anti-HBV therapy does not directly affect the hepatic viral reservoirs (cccDNA). Because HBV integrates in to the host genome, it is not known whether treatment will prevent the development of HCC, especially if cirrhosis exists at the time anti-HBV treatment is initiated. HCC has been described in persons with a remote history of HBV who did not have evidence of active viral replication (Okoshi et al., 2002) and the HBV X gene can be detected in liver tissue from persons with HCC who are HBsAg and HBV DNA negative (Higashi et al., 2002).

The correlates of successful therapy are not clearly delineated, but markers of treatment successful include improvement in liver histology, normalization of hepatic transaminases, substantial diminution in HBV viral load, and, in those with circulating HBeAg, the loss of e antigen with the development of anti-HBe ("HBeAg serocoversion"). Sustained loss of HBsAg is considered by
some to be a “complete response” (Lok et al., 2001) although a decline in HBV viral load correlates with response, no target HBV viral load has been established, and HBV DNA assays are not standardized. The newer PCR based methods have a lower sensitivity limit of $10^2$ to $10^3$ copies/ml compared with $10^5$ for older assays (Lopez et al., 2002).

For persons without HIV, the AASLD recommends that persons with HBeAg-positive chronic hepatitis B with evidence of liver disease (ALT two times normal or necroinflammation on biopsy) and those with HBeAg negative chronic hepatitis B with HBV-DNA $10^5$ copies/ml should be considered for treatment (Lok et al., 2001). This value for the HBV-DNA cutoff is arbitrary, but it is endorsed by the AASLD and seems reasonable based on the current state of knowledge. The same criteria can be applied to persons with HIV infection, with the caveat that the state of the HIV disease should receive priority in terms of evaluation and treatment because morbidity and mortality from HIV occurs more rapidly. Although the AASLD does not routinely recommend liver biopsy for the evaluation of chronic hepatitis B, a biopsy may support a decision to delay therapy in order to spare HAART toxicity or to prevent resistance in the HIV-HBV coinfected person. The three currently approved therapies for chronic hepatitis B are interferon-α, lamivudine, and adefovir.

2.3.6.1 Interferon-α

Interferon-α, an agent with antiviral and immunomodulatory effects, is approved at a dose of 5 million units subcutaneous (SQ) daily or 10 million units SQ three times per week. For persons who have circulating HBeAg, 16 weeks of interferon-α is recommended, whereas those without circulating HBeAg require a minimum of 12 months (Lok et al., 2001). Interferon-α is most effective in persons
with high ALT (>2 ULN), lower HBV-DNA (<2.8 x 10^7 copies/ml or 100 pg/ml) and circulating HBeAg (Brook et al., 1989 and Perrillo et al., 1990). They found a 50% response rate in those with HBV-DNA <2.8 x 10^7 copies/ml and a 7% response with levels >5.6 x 10^7 copies/ml. However, a reproducible, discriminatory level of HBV-DNA above which response to interferon-α is unlikely has not been determined. The frequent side effects of interferon-alfa, including flu like symptoms, psychiatric effects, and bone marrow toxicity, have limited its use. Its advantages are higher HBeAg seroconversion rates and limited treatment duration (16 weeks) in persons who have detectable HBeAg. In HIV infected individuals it has the additional advantage of not engendering HIV resistance. Interferon-α is contraindicated in decompensated liver failure or sepsis (Perrillo et al., 1995).

A meta-analysis of randomized trials, which included 837 persons with an ALT >2X ULN and circulating HBeAg, demonstrated a 33% HBeAg seroconversion rate in persons given 16 weeks of interferon-α compared to 12% in untreated controls. A durable response of HBeAg clearance is seen in 80 to 90% of persons. However, HBV DNA and HBsAg often remain detectable (Krogsgaard et al., 1998). Clearance of HBeAg and not HBV DNA has been correlated with improved clinical outcome (Fattovich et al., 1997).

Studies of interferon-α therapy in HIV-HBV coinfected individuals are limited but suggest a decreased response compared with those without HIV infection. In meta-analysis, 55 of the 837 individuals were coinfectcd with HIV, and they showed a 38% decreased response compared with HIV uninfected persons (p=0.02). In addition, the immune status (CD4) count and the ALT values of these patients are not known. Two small, randomized studies and five cases series have treated 98 HIV-HBV coinfected patients with interferon-α with a 14.3% HBeAg
seroconversion rate (Wong et al., 1995). In the two randomized studies, none of the 14 untreated controls experienced HBeAg seroconversions (Wong et al., 1995 and McDonald et al., 1987). Although data extrapolated from these studies collectively suggest a decreased response to interferon-α in HIV–infected patients, these studies did not consider the degree of immunosuppression, and interferon-α has not been evaluated in the era of HAART. Given that interferon-α is an immunomodulating agent, it seems reasonable that those with a more robust immune system (CD4>500) would have a greater likelihood of response. ALT levels, which correlate with response, were not considered, so coinfected individuals with high ALT values may have an equivalent response to the individuals not infected by HIV.

A pegylated interferon-α that permits once weekly dosing and produces more constant levels is more efficacious than standard interferon-α is for treating chronic hepatitis C. Preliminary observations suggest that this may also be true for the treatment of chronic hepatitis B in individuals not infected by HIV especially those with lower ALT values (Fried et al., 2002).

In summary, although the response to standard interferon-α in HIV-HBV coinfected patients may be lower than it is in persons not infected by HIV, the data suggest that those most likely to benefit have an elevated ALT (> 2 x ULN), low HBV DNA (< 2.8 x 10⁷ copies /ml), and relatively preserved immune function (CD4>500). Thus, in the HIV-HBV coinfected individual with these characteristics and in whom HIV therapy is not indicated, pegylated interferon may be the preferred option because it spares both HAART toxicity and HIV resistance to antiretroviral medications.
2.3.6.2 Nucleoside and nucleotide analogues

Nucleoside and nucleoside analogues inhibit HBV replication by competing with the natural nucleoside triphosphates for incorporation into viral DNA. Lamivudine and adefovir are the approved nucleoside and nucleotide analogues, respectively, for the treatment of chronic hepatitis B.

Lamivudine, which also has activity against the HIV reverse transcriptase gene, was the first oral anti-HBV agent approved at a dose of 100 mg daily and has gained widespread use because of its ease of administration and its tolerability. In HIV-infected patients, lamivudine should be administered at 150 mg twice daily as a component of a HAART regimen in order to prevent the rapid emergence of HIV resistance. It is effective against HBeAg-positive and-negative disease; however, the treatment duration differs. For HBeAg-positive persons, treatment is for 6 months after HBeAg seroconversion or 1 year, whichever comes later. For HBeAg negative persons, treatment is longer but the optimal duration of therapy is unknown. The HBeAg seroconversion rate in large studies is between 16 and 18% and correlates with ALT levels (Lai et al., 1998 and Dienstag et al., 1999). In the Asian trial, those with ALT >5 X ULN had a 64% HBeAg seroconversion rate compared with 26 and 5% among those with an ALT >2 X and <2X the ULN, respectively (Chien et al., 1999). The durability of the response in HIV-uninfected patients varies between 63 and 80% (Dienstag et al., 1999 and Song et al., 2000) and may be more likely if therapy is continued for more than 6 months after HBeAg seroconversion (Pil et al., 2001). In those with HBeAg negative disease, biochemical, histological, and virological responses occur, but the release rate is 90% on discontinuation of lamivudine. Chronic dosing in this setting has not been adequately studied. Studies of HIV-HBV coinfected subjects show rates of HBeAg seroconversion of 22 to 28% (Benhamou et al., 1996) that appear comparable to the HIV-uninfected patient.
As in the treatment of HIV, lamivudine therapy is limited by the selection of lamivudine-resistant HBV strains, which may develop more rapidly in subtype adw than in ayw. As in HIV, the mutations usually occur in the YMDD motif of the catalytic domain of the polymerase gene and change the methionine at position 204 to either isoleucine or valine (Stuyver et al., 2001). A recent report of primary infection with this mutant virus demonstrates the ability of it to be transmitted (Thibault et al., 2002) in persons uninfected by HIV, resistance increased from 14% at year 1 to 38, 49, and 66% at years 2, 3 and 4, respectively (Lok et al., 2001). The development of resistance in HIV-HBV coinfected persons may be greater and is estimated at 20% per year (Benhamou et al., 1999). The HBV-DNA level may predict resistance after 6 months of the initiation of treatment (Yuen et al., 2001).

The emergence of mutants is usually clinically detected by flares in liver disease, manifested by ALT elevations and reappearance of HBV-DNA. If the mutation appears, lamivudine should be continued in the coinfected patient unless it is determined to effective HIV therapy because severe, even fatal, rebound hepatitis may occur on discontinuation of lamivudine in those who have detectable HBV-DNA (Lim et al., 2002). If lamivudine is discontinued, careful monitoring of ALT and HBV-DNA is mandatory. Despite resistance, HBeAg seroconversion has been reported in 25% of patients who continue lamivudine after the appearance of the mutants (Liaw et al., 2000), but long-term lamivudine for isolated chronic hepatitis B after the development of resistance is a controversial area.

In summary, lamivudine is a potent anti-HBV drug, but it should not be used as a single agent in the coinfected patient because of the rapid emergence of HIV and HBV resistance mutations. Its use in the coinfected patient naïve to therapy for both viruses will likely be in combination with either adefovir or tenofovir along with a potent antiretroviral regimen. Prior to starting a HIV-infected patient on
lamivudine, one must be aware of patient's HBV status in order to not limit future options for HBV therapy.

Adefovir is approved at a dose of 10 mg daily for chronic hepatitis B treatment and is a active against lamivudine-resistant virus either alone or in combination with lamivudine (Peters et al., 2002) after 48 weeks of therapy, HBeAg seroconversion occurred in 12% of patients (Marcellin et al., 2003) and seemed to be inversely correlated with hepatic levels of cccDNA (Werle et al., 2002). Notably, adefovir appeared to decrease the hepatic cccDNA levels despite the general belief that nucleoside and nucleotide analogues do not decrease the pool of cccDNA (Werle et al., 2002). HBV resistance mutations to adefovir are uncommon. A recent report demonstrated a novel mutation in the HBV polymerase (N236T) in 2 of 124 patients (1.6%) receiving 96 weeks of adefovir dipivoxil. This mutation reduced susceptibility to adefovir 5- to 23-fold in vitro, but there was no cross-resistance to lamivudine (Xiung et al., 2003). In a cohort of 32 HIV-HBV coinfected patients treated for 48 weeks with adefovir, the response rate is similar with an HBVDNA decline of $-4 \log_{10}$ copies /ml and HBeAg seroconversion in 2 patients (Xiung et al., 2003).

Tenofovir, a nucleotide analogue similar to adefovir, is not yet approved for the treatment of chronic hepatitis B, but data suggest that declines in HBV-DNA are similar to those for adefovir in lamivudine-resistant and wild type HBV (Nunez et al., 2002). For the HIV-HBV coinfected patients, its advantage is that it is also active against HIV.

In summary, adefovir and tenofovir are effective against both lamivudine-resistant and wild-type chronic hepatitis B, and resistance develops slowly. Thus, use of these agents as single agents should be done cautiously and is probably best
reserved for the patient with lamivudine–resistant HBV who has evidence of moderate to severe fibrosis who has evidence of moderate to severe fibrosis on liver biopsy. Adefovir could also be cautiously used in the patient with moderate to severe fibrosis who does not need HIV treatment and is not a candidate for interferon therapy because the dose of adefovir may be too low to exert mutation pressure on the HIV reverse transcriptase and thus may not select for adefovir-resistant HIV, but there are limited data to support this (Delaugerre et al., 2002).

2.3.6.3 Summary of current therapy options

The treatment of chronic hepatitis B in the HIV-infected patient is complicated and needs to be individualized. In considering the need for therapy, the HBeAg status is the first discriminatory point in the algorithm. Those who are inactive carriers (HBeAg negative with <10^5 copies/ml of HBV-DNA and normal ALTs) do not need treatment but should have periodic monitoring of ALT, aspartate aminotransferase, HBV-DNA every 4 to 6 months and of AFP or ultrasound, or both, for HCC. If ALTs become elevated for a sustained period of time, then a liver biopsy may be warranted. Those who do not have circulating HBeAg but have >10^5 copies/ml of HBV-DNA should be evaluated as if they are HBeAg positive.

The therapy options for the HBeAg positive can grouped based on their need for HIV therapy. The most straightforward is the coinfected patient who needs treatment for both HIV and HBV and has not received therapy for either. Provided they have not been infected with a resistant virus, lamivudine and tenofovir should be active against both and provide a strong backbone to a HAART regimen. For the patient who does not need HIV treatment but has chronic hepatitis B, a liver biopsy is useful to assess the severity of the liver disease. If there is minimal liver disease, then close monitoring for liver disease and HCC is needed. If HBV therapy is
needed, then pegylated interferon for 16 weeks is a good first option if the CD4 is >500, the ALT is >2 x ULN, and the HBV DNA is <2.8 x 10^7 copies/ml. This option spares both HAART toxicity and selection of HIV – and HBV – resistant viruses. Those without these characteristics or who fail pegylated interferon can be treated with HAART, including lamivudine and denofovir or potentially with adefovir alone. The most complicated situation is the HIV-infected patient who has been on lamivudine for years and has detectable HBV-DNA signifying the presence of lamivudine-resistant HBV. If the patient is still on lamivudine, it should be continued if possible, because discontinuation may be harmful. In this situation, a liver biopsy is preferable to assess the need for HBV therapy. If therapy is needed, adefovir or tenofovir are the favored options to add to the HAART regimen. Pegylated interferon is also an option if they have favorable characteristics; however, this has not been studied in lamivudine-resistant HBV. If therapy is not needed, then one should consider sparing adefovir and tenofovir for future combinations unless tenofovir is needed to treat the HIV disease.

2.3.6.4 Future therapeutic directions

Several other agents are in development but appear promising. Entecavir is active against the HBV polymerase and a 48-week course produces a – 5.11 log HBV-DNA decline against lamivudine – resistant HBV without evidence of resistance mutations (Chang et al., 2002). Emtricitabine (FTC), a fluorinated derivative of lamivudine, has activity against HIV and HBV. A study of 96 weeks of FTC shows HBeAg loss of 51% and anti-HBe seroconversion in 29% of subjects. As expected, resistance develops but at a slower rate than to lamivudine (Gish et al., 2002). Telbivudine (L-dT) is an L-nucleoside analogue that is very potent and
appears to show greater efficacy in reducing HBV-DNA levels than lamivudine does (Lai et al., 2002).

Combination therapy for chronic hepatitis B is attractive because it has the potential for greater potency while preventing the development of resistance. The only combination that has been studied (although not extensively) is interferon-α and lamivudine, which does not appear to be substantially better than the individual medications alone (Barbaro et al., 2001). However, it is anticipated that we are entering the era of combination nucleoside analogue therapy for the treatment of chronic hepatitis B. Those treating HIV-HBV coinfected patients may be at the forefront of this movement because the combination of tenofovir and lamivudine provides a potent combination against both viruses, especially in the patient who is naïve to therapy.

2.3.6.5 Liver transplantation

For end-stage liver disease, transplantation is an option. The HBV infection rate in the transplanted liver is very high but can be reduced by the posttransplant administration of hepatitis B immunoglobulin and lamivudine (Markowitz et al., 1998). For the HIV infected patient, liver transplantation is still experimental, so patient should be referred to centers with an active HIV transplant program.

2.4 HIV and HCV

HIV/HCV coinfection is increasingly recognized as a growing public health problem. Early in the HIV/AIDS epidemic most people with HIV were
expected to die from AIDS, and less attention was devoted to other long term conditions. Because chronic hepatitis C progresses so slowly, many HIV positive people who were infected with HCV in the 1970s or 1980s are only now beginning to develop advanced liver disease. As improved HIV treatment has reduced mortality due to 'Opportunistic Illness' (OIs), liver failure – often related to chronic viral hepatitis- has become a major cause of hospitalization and death in people with HIV/AIDS. In some recent studies liver failure due to HCV was the leading cause of death.

2.4.1 The “twin epidemics”

HIV and HCV display some common biological features both are RNA Viruses: HCV belongs to the Flaviviridae family, and HIV to the Retroviridae family. Flaviviruses have a single -RNA strain, while Retroviruses have a double RNA strain. The life cycle of both viruses also have some differences. The HIV-RNA, transcribed to DNA by the reverse transcriptase (RT), integrates in the infected cell’s genome, constituting the integrated provirus; this integration is the cause of the irreversibility of HIV infection. In contrast, the HCV genome does not integrates into the cell’s genome, and the replication of that virus takes place in the liver cell’s cytoplasm. This non-integration makes it easier to eradicate HCV, and hence to cure the infection.

Another characteristic of both viruses is the large heterogeneity of their respective viral genomes (Brechot et al., 1994 and Simmonds et al., 1995) producing a variety of stereotypes and so-called quasispecies, genetic variants around a ‘master sequence’ (Domingo et al., 1997). This genetic variability is the consequence of a high mutation rate in RNA viruses, derived from the high rate of
replication errors, a direct result of the non proofreading ability of their polymerases (RT for HIV and a RNA polymerase RNA dependent for HCV) This inability of these enzymes makes it, compared to DNA Polymerases DNA-Dependent, highly error prone. The mutation rate for RT is $1 \times 10^{-4} - 10^{-5}$ nucleotides, and it means a mutation at each completely transcribed HIV genome. Similar numbers have been described for HCV (Domingo et al., 1997). In this virus there is variability among the nucleotide sequence of up to 34% among the most distant variants (Simmonds et al., 1995). This genetic variability is higher for HIV, due to probable eventuality of recombination among the two RNA chains of the virus, by a phenomenon known as “strand choice” (Domingo et al., 1997). This variability allows both viruses to develop a better “fitness” in the presence of some environmental circumstances, and to avoid either the immune system or the pharmacological pressure. The most heterogeneous region for the viruses is the envelope-codifying region, probably related to the presence of the immune system over the virus. The diversity of the region in HCV differentiates 6 genotypes (1 to 6), divided also into several subtypes (Simmonds et al., 1995) are recognized: M (main) with ten subtypes inside, O (Outlier) and the recently described group N (Simon et al., 1998).

The interest is classifying the virus species is not exclusively academic or taxonomic, but also important for clinical practice. Some variants of the same virus might have either a particular target cell, or a different sensitivity to antiviral drugs, or are more often transmitted in a certain fashion. In this way, HCV subtype 1b has a worse response to αIFN than all the other subtypes (Zeuzem et al., 1997). Besides, it is known that some particular subtypes are predominant in certain population, or in a geographic area, or even among patients belonging to a particular risk group (i.e., HCV subtype 3 in European IVDVs) (Nousbaum et al., 1994). The availability of some amplification techniques that allow one to detect and quantify the number of
viral particles circulating in the blood, has yielded some information about other analogies between HIV and HCV.

Some recent reports have shown a similar replications kinetic for both viruses, with an average life-span of a virion in the blood of less than 4 hours, and a surprisingly high rate of turnover in the viral population (Lam et al., 1997 and Neumann et al., 1998) One trillion ($10^{12}$) virions are produced and cleared daily. Furthermore, contrary to previous suggestions, the viral load in patients infected by HCV or HIV remains almost stable over time, with only small changes, usually not higher than $0.5 \log^{10}$ (Henrard et al., 1995 and Yoshimura et al., 1997). In spite of this, there are important variations among different patients. Some groups have reported a higher HCV viral load in patients coinfected with HIV, compared to those not coinfected (Eyster et al., 1994 and Cribier et al., 1995). Moreover, HCV viral load shows an inverse correlation with the level of immunodeficiency in HIV-infected patients, as reflected by the number of CD4 cells (Cribier et al., 1995).

2.4.2 Why is HIV – HCV coinfection important

The introduction of highly active antiretroviral therapy (HAART) for HIV in the mid-1990s has caused a sharp drop in the number of deaths resulting from AIDS. This means that people with HIV are living longer. Therefore, if they are coinfected, the complications from HCV have more time to develop. These complications (cirrhosis, liver cancer, end-stage liver disease) generally develop over 20-30 years. Liver disease from HCV is now the leading non-AIDS cause of death in the U.S. in coinfected individuals with HIV (CDC-2002).

Treatment for each disease is complicated, expensive, and has side effects. This poses difficult issues for patients who are living with both HIV and
HCV. Finally, co-infection is important because it has a disproportionate impact on certain communities, including those in prison and communities of colour.

2.4.3 Epidemiology of HIV and HCV coinfection

There are about 150 million chronic hepatitis C (HCV) carriers throughout the world with an estimated global prevalence of 3% (range 0.1 - 5%) (EASL, 1999). In the United States nearly 2% of the population is infected with hepatitis C virus (HCV) (NIH, 1997). Symptomatic acute infection occurs in an estimated 1-3 cases/100,000 persons annually but the actual incidence of new HCV infection is higher as the majority of cases are asymptomatic. Following exposure to hepatitis C, approximately 85% of patients develop chronic infection (NIH, 1997 and Hoofnagle et al., 1997). Chronic HCV infection can lead to cirrhosis and hepatocellular carcinoma, accounting for about 8,000-10,000 deaths annually in the United states alone (NIH, 1997). Additionally, HCV-induced end-stage liver disease accounts for about 30% of liver transplants in industrialized countries, presenting a significant burden on health care costs (EASL, 1999). Coinfection with HCV in HIV-infected individuals is common, presumably due to the shared route of transmission of these viruses. The prevalence of HCV infection among all HIV-infected individuals can be as high as 40% but this prevalence varies substantially among different risk groups (Dieterich et al., 1999). In most series the prevalence of HCV among HIV-infected intravenous drug users (IVDU) is 50-90% (Huemer et al., 1990). In a large cohort of 3,048 HIV-infected subjects from the Euro SIDA study, 33% were anti-HCV antibody positive and more than 75% of IVDUs in this population were coinfecte. Among hemophiliacs, coinfection with HCV is found in up to 85% of individuals with HIV (Dieterich et al., 1999). However, the prevalence of HCV in HIV-infected homosexual males is similar to that observed in HIV-negative homosexual males at 4-8% (Bodsworth et al., 1996). Although the rate of
sexual transmission of HCV is low (<5%), this rate may be increased in the setting of coinfection with HIV (Thomas et al., 1996). In a Spanish series of 294 female prostitutes who denied IVDU, 5.8% were seropositive for HCV, and coinfection with HIV was independently associated with hepatitis C seropositivity. Similarly the rate of mother-to-infant transmission of HCV increases in the presence of HIV, presumably due to high levels of HCV viremia observed in these individuals (Toyo et al., 1997; Thomas et al., 1998 and Zanetti et al., 1999). Interestingly, there is an increased rate of vertical transmission of HIV in the presence of HCV. In a large prospective U.S. series of 487 HIV-positive pregnant women, of whom 161 were positive for HCV-RNA, the rate of mother-to-infant transmission of HIV was 26% in HCV-positive compared to 16% in the HCV-negative mothers (OR 1.82, p=.01) (Hershower et al., 1997). HIV/HCV coinfection is common with an especially high prevalence among IVDUs. HIV coinfection may enhance the sexual and vertical transmission of HCV.

2.4.4 Impact of HIV on the course of HCV infection

HCV induced liver disease can be progressive with cirrhosis developing in up to 20-30% of individuals over a 10-20 year follow-up (Hoofnagle et al., 1997). HIV coinfection has been associated with a more rapid progression of liver disease as well as a higher prevalence of cirrhosis (Garcia-Samaniego et al., 1997 and Benhamou et al., 1999). In parentally acquired HCV infection (blood transfusion recipients or IVDUs), there is a higher incidence of cirrhosis in HIV-positive compared to HIV-negative individuals within the first 15 year of follow up (15-25% vs 2.6-6.5%, respectively, p<0.05) (Sanchez-Quijano et al., 1995 and Soto et al., 1997). Additionally, the estimated interval from HCV infection to cirrhosis may be significantly shorter in the HIV-infected individuals (7 Vs. 23 years, p<0.001) (Soto et al., 1997). A recent study compared a cohort of 122 HIV/HCV coinfected
individuals with 122 HIV-negative, HCV-infected patients. HIV seropositivity 
(p<0.0001), alcohol consumption of >50g/d (p=0.0002), age at infection greater than 
25 years (p<0.0001) and CD4 T lymphocyte counts less than 200 cells/mm³ 
(p<0.0001) were associated with an increased rate of liver fibrosis progression 
(Benhamou et al., 1999). Among the coinfected individuals, alcohol consumption, 
low CD4 counts, and higher age at HCV infection were independent predictors of 
fibrosis progression (Benhamou et al., 1999). Although HIV/HCV coinfection 
results in a more rapid progression of liver disease, its effect on mortality requires 
further assessment. Some have found a higher rate of mortality from liver-related 
diseases in the coinfected patients, (Di Martino et al., 2000) yet others have not 
shown any effect on survival (Wright et al., 1994 and Macias et al., 1998).

HIV/HCV coinfection is still poorly understood, but recent research has 
shed light on how the two viruses interact. A review of the pathophysiology of 
HIV/HCV coinfection suggests a strong cell mediated immune response involving 
both CD4 and CD8 cells is required to keep HCV under control. A strong immune 
response also appears necessary to enable successful HCV treatment with interferon. 
In people with HIV, the immune response may be compromised, making it less 
likely that infected persons will clear HCV and allowing HCV to replicate more 
rapidly.

Much of the liver disease related to hepatitis C is caused not by the virus 
itself, but rather by the immune system's response to HCV. Thus, it might expected 
that people with compromised immune system would mount a weaker immune 
response that causes less liver tissue damage. However, research indicates that the 
opposite seems to be the case.
A HCV infection was misinterpreted as autoimmune type of chronic active hepatitis (Berk et al., 1990) as it was diagnosed based on non-specific criteria such as a 10 fold increase in serum transaminase activity, a two fold increase in the Ig-G concentration, the presence of auto-antibody and dense lymphoma cellular infiltrate in the liver but later it was confirmed to be severe hepatitis due to HCV infection since it is responded well to two weeks of IFN treatment and subsequently confirmed by the detection of anti-HCV antibodies. In addition to that this report supported the observation that chronic HCV infection can progress rapidly where here is coinfection with HIV, as described earlier.

A study on the correlation of hepatitis C virus antibodies with HIV-1 seropositivity in IVDUs revealed that almost twice as many HIV positive drug addicts show antibodies to HCV as the HIV negative IVDUs do (Huemer et al., 1990). On the other hand, seropositivity for hepatitis B virus is almost the same in both groups. This could indicate that the infectivity of HCV is lower than that of hepatitis B virus or that other mechanisms such as immunosuppression (Libanore et al., 1988) and the more frequent secondary infections in anti-HIV positive individuals may play a crucial role in the acquiring of HCV seropositivity.

Analysis of serum specimens from 111 human immunodeficiency virus type-1 (HIV-1) infected and 183 HIV-1 seronegative patients for antibodies to HCV, and HBV revealed that anti-HCV antibodies were found in anti HIV-1 positive homosexual men (14%) and anti-HIV-1 negative heterosexual persons (8%), but not in HIV-1 seronegative homosexual men. The study concluded that the prevalence of anti-HBc antibodies was much higher than that of anti-HCV antibodies among the homosexual men suggesting that hepatitis B is more readily spread than HCV.
The frequency of HCV transmission with HIV, was five times higher when HIV also transmitted, suggesting that HIV may be a cofactor for the sexual transmission of HCV (Elaine Eyster et al., 1991). This report found out 2.6% of cases was anti-HCV positive when a cross-section of 194 female sexual partners of HCV infected men was studied. It was similar to the report of homosexual men. It also supports the finding of Alter et al., 1989 which suggested that heterosexual transmission may play an important role in the spread of non-A& non-B hepatitis. According to Boyer et al., (1992) the response of patients with CHC with HIV infection and tolerance of recombinant IFN-alpha were not different from patients with CHC infection without HIV infection. This was observed by histological examination of liver but response to IFN-alpha was good when ALT of serum was seen.

The HCV induced liver failure occurs significantly in HCV/HIV coinfected adults who had been infected for ten or more years. They have also suggested that HIV or its therapy may accelerate the liver failure in HCV positive adults with hemophilia. The same results were also reported by others (Martin et al., 1989 and Filippo et al., 1991).

In one study, out of 91 cases 8 cases were of HCV and HIV positive AIDS free persons developed liver failure. In which, the level of HCV-RNA both in HIV negative and HIV positive hemophilic the mean base line level of HCV-RNA were similar in two groups but increased over the next 5 to 12 years in both groups, the increase was 8 fold greater in HIV positive than in HIV negative groups. HCV-RNA level increased twice in HCV/HIV coinfected individuals who develop the liver failure compared to those who did not (Elaine Eyster et al., 1993).
A strong negative correlation between HCV-RNA levels and CD4 counts was observed, which suggests possible HIV induced immunodeficiency and in turn an increased HCV replication. This report included the possible reactivation of HCV in the immune deficient host and also the transmission of HCV along with HIV to the sexual partners. Hepatocellular damage by HCV may be as that of HBV mediated by the immune reaction to infected hepatocytes, however other reports suggest that HCV may be cytopathic to Liver cells. Jarvis et al., 1994 revealed the effect of HIV in the generation of distinct genotypes of HCV, they have concluded genotype change of HCV is more prevalent in HIV positive cases. Reexposure of infected HCV seropositive Chimpanzees to HCV led to the reactivation of clinical hepatitis (Prince et al., 1992).

Reinfection also occurs with identical virus variants to the one used for the initial infection which indicates HCV infection does not elicit a protective response even against the homologous strains (Prince et al., 1992). Other reports (Prince et al., 1992 and Lai et al., 1994) suggested that multiple exposures due to long term use of non virus inactivated concentrates may have led to sexual episodes of re-infection and possibly pathogenic variants.

Wright et al., 1994 concluded that hepatitis C virus coinfection, like HBV does not influence the survival of HIV infected patients irrespective of manifestation of AIDS. So also HCV infection is more common in non-IVDU, HIV positive patients than in voluntary blood donors suggesting the sexual transmission of HCV. Conversely Sonnerborg et al., 1990 concluded that HCV coinfection is more common among HIV seropositive IVDU than in HIV positive homosexual men and heterosexual contacts.
The HCV was proposed to have no influence on clinical and immunological progression of HIV disease (Dorrucci et al., 1995). It was conformed with other reports (Quan et al., 1993) suggesting that HCV coinfection does not accelerate the progression of HIV disease. In 1994 Soto et al., reported that HCV infection may be sexually transmitted but with low efficiency, particularly when compared with HIV infection and the rate of sexual transmission could be increased in the presence of coexistent HIV infection in the index case.

In one study on the morphological lesion of the liver in HCV/HIV positive patients, mainly intravenous drug addicts from a group of patients not infected with HIV. The liver damage was significantly more severe in HCV-positive / HIV negative patients and in HCV / HIV positive when the CD4 cells count was higher than 400 cells/mm³, whereas less severe liver histology in HCV/HIV-coinfected patients correlated with the drop in the CD4 lymphocyte count and they have concluded that liver lesions in hepatitis C may largely depend on immune mediated mechanisms.

In the HIV/HCV coinfectected patients, the HCV-related factors (pretreatment serum HCV-RNA levels and genotype) have a more marked influence than patient-related factors on the response to IFN therapy. Because pretreatment serum HCV-RNA levels and HCV genotype are independent factors, it may help to predict treatment outcome (Peignoux et al., 1995).

In 1995 Fiore et al., strongly suggested that semen should not be considered as an important vehicle for HCV transmission. In fact, even in HCV viremic HIV-1 infected individuals, HCV is usually absent in semen or present only in trace amounts. The particular life-style of high risk groups such as drug addicts combined with a lack of preventive measures for virus transmission through
sexual contact is likely to explain the increased rate of HCV transmission in individuals coinfected with HIV-1 and HCV.

Distinction of HCV genotypes is important since the outcome of chronic infection and response to antiviral therapy with infection correlates with the genotype. Genotype 1, especially subtype 1b, has been associated with more severe chronic liver disease and poor response to IFN therapy. Berg et al. (1997) observed statistically significant higher HCV-RNA titers in hemophiliacs infected with HCV genotype-1 compared to those infected with other genotypes (p<0.01). No relationship was found between the presence of HIV coinfection and viral load of HCV-RNA. They also observed that there was no evidence that HCV infection had a more severe outcome in HIV-positive patients who had been infected with HIV and HCV more than ten years ago, even in those with very low CD4 cell counts. Finally they concluded that a large viral load (HCV-RNA) is associated with HCV genotype 1 infection and that HIV coinfection has clear effect on the intensity of HCV replication.

In HIV/HCV coinfected patients, HCV-RNA levels had inverse correlation with CD4 counts but no correlation with serum aminotransferase levels. In addition to that HCV virus replication appears to be increased in patients with severe immunodeficiency secondary to progressive HIV infection, and the absence of correlation between HCV- RNA and serum ALT level suggesting that HCV is not directly cytopathic (Ghany et al., 1996).

Sherman et al., 1996 clearly explored the possible envelope variants of HCV in HCV & HIV coinfected patients compared to HCV alone infected patients by analyzing nucleotide sequence variability in the E2 / NS1 HVR-1 of the HCV genome. Variability was observed in the sequence codes for aminoacids from 384 to
414 specifically the greatest variability was observed at amino acids 386,397,400,402,405,407 and 414. Nonsynonymous clonal variation resulting in alteration of putative antigenic sites within in HVR clones with unique predicted antigenic domain observed more frequently in HCV / HIV coinfected patients were consistent with increased sequence variability in turn suggests an accumulation of envelop variants in HCV / HIV coinfected patients, may cause resistance to IFN in the patient group studied.

A study on HCV coinfection with HIV revealed that HCV-RNA levels were strongly increased in the case of HIV infection, but HCV load is not linked to the immunosupression induced by HIV and also suggests the absence of direct interaction between HIV & HCV but other indirect factors present in HIV infected patients may play a role in the enhancement of HCV replication (Cribier et al., 1995). In another study of HIV and HCV coinfection in hemophiliacs revealed that HIV may influence the progression of liver failure due to progressive immune dysfunction. HIV positive patients with CD4 counts below 400 cells /\\mu l shown significantly less portal inflammation and piecemeal necrosis indicating that liver disorder with HCV may depend on immune mediated mechanism.

HIV and HCV coinfection paved the way for rapid progression of clinical complications. Molecular analysis revealed the interactions between HIV and HCV and between HCV subtypes 1a and 1b. Ineffective immuneresponse was the result due to persistence and sequence conservation of the HCV HVR-1 variants. Mutated HV-1 (hyper variant) was a dominant strain in all patients with peak HCV-RNA titer. The higher rate of replication during the 1st phase of infection exposed variants HV-1 to the host immune pressure subsequently have brought about the selection of HVR-1 mutation. Conversely variants in advanced stages of HIV infection had lower evolutionary pressure; so lower HVR-1 mutant was selected (Mazza
The HIV/HCV coinfected individuals infected with HCV type-1 progressed HIV disease more rapidly than those infected with other types of HCV.

HIV plays a vital role in modifying the natural history of chronic parenterally acquired HCV with an unusually rapid progression to cirrhosis and HIV-related immunodeficiency may be a determinant of higher hepatitis C viremia levels and more severe liver damage also (Soto et al., 1997). HCV subtype influencing the liver damages in patients with CHC 1b subtype closely associated with more severe liver pathology. Higher degrees of piecemeal necrosis and fibrosis occur in HIV/HCV coinfected patients with genotype of HCV 1b. In the HIV/HCV coinfection HIV infection, which causes an enhancement of HCV-RNA levels is an independent factor associated with more aggressive forms of liver pathology (Garcia-Samaniego et al., 1997).

The anti-HIV drugs like PIs (protease inhibitor) may temporarily worsen the HCV status with the improvement of HIV parameters (Rutschmann et al., 1998). The HIV infection worsens the course of chronic HCV in HIV/HCV coinfected IVDU and also excessive alcohol drinking appears to be a crucial negative factor as a result the alcohol withdrawal as an integral part of the therapy (Pol et al., 1998).

The HCV must be treated as an opportunistic pathogen in HIV disease as they have observed more Progressive Liver Disease (PLD) occurrence in HIV/HCV co infected group. PLD occurrence was higher in subjects with severe AIDS defining immuno deficiency moreover PLD has caused a faster progression to AIDS (Lesens et al., 1999). Hepatitis may reflect the restoration of anti-HCV immune response in HIV/HCV coinfected patients following treatment with potent ART when compared with HCV replication or a hepatotoxic effect of ART (John et al., 1998).
Both HIV and HIV induced immunodeficiency enhance HCV replication among HCV seroconverters (Beld et al., 1998). They have concluded that (i) HIV infection leads to enhanced HCV replication (ii) an inverse relationship between CD4 cell counts and HCV-RNA levels is seen in HIV infected individuals and (iii) there is an inverse relationship between CD4 cell counts and HCV-RNA levels among HIV negative individuals, suggesting that HCV replication is under the control of immune system.

In HIV/HCV coinfected patients, alcohol consumption (>50 g/d) CD4 count (<200 cells/μl) and age at HCV infection (<25 years old) (p<0.0001, respectively) were associated with a higher fibrosis progression rate than in control patients with HCV alone infected (Benhamou et al., 1999). Hepatitis C virus (HCV) has emerged as a major pathogen among patients with HIV. Morbidity and mortality were compared among 263 patients with HIV alone, 166 patients with HIV and HCV, and 60 patients with HCV alone. No difference in HIV loads and CD4 cell count was observed between the HIV and HIV/HCV groups. Alanine aminotransferase levels were higher (52U/l versus 35U/l; p<0.02) among coinfected patients than they were among patients with HIV alone. Liver decompensation developed in 10% of patients with HIV/HCV coinfection (Monga et al., 2001). In contrast, no liver related deaths or decompensation occurred in patients without coinfection (p<0.05). Of the patients with HIV alone, 7% died, compared to 11% of coinfected patients (p<0.02) 47% of deaths in the latter group were due to liver related causes. Finally they concluded that HCV infection causes increased morbidity and mortality in patients with HIV infection.

The asymptomatic HIV coinfection alters HCV specific cytokines response with a greater production of proinflammatory type-1 cytokines and also type-1 cytokines may be modified by an increased production of type-2 cytokines in
the CD 30 subset and finally the modification of this cytokines patterns may contribute to the adverse natural course of HCV in HIV coinfection. The HIV/HCV coinfected patients with low CD4 cell count and presence of many fibrous septa had an association and it was independent of HIV infection and other factors. This in turn suggests that HIV infection–induced CD4 depletion is independently associated with the severity of liver fibrous in chronic HCV infection. Hence, the early combination ART might interrupt the vicious cycle between CD4 cell depletion and accelerated progression of liver disease in patients with HIV/HCV coinfection (Puoti et al., 2000).

The HIV/HCV coinfection in IVDU may play a pivotal role in the mortality among HIV-1 infected patients possibly through impaired CD4 cell recovery in the HCV/HIV seropositive patients receiving potent ART (Greub et al., 2000). The HCV-RNA load was independently associated with HIV-1 disease progression in HIV/HCV coinfected patients. In one study Daar et al., (2001) suggested a possible interaction between these two RNA viruses that often results in chronic infection in coinfected individuals.

The histological severity of chronic hepatitis C in the patients coinfected with HIV did not differ in relation to CD4 cell count while other study showed a significantly lower degree of portal inflammation and piecemeal necrosis in HIV positive patients with < 400 cells (Romeo et al., 2000). Considering HCV-RNA load, histological activity index, response to IFN therapy, and liver related death as parameters to study the impact of HIV on chronic hepatitis C infection, the higher serum HCV-RNA level (p=0.012) seems to be higher total Knodell’s score (p=0.01) and poorer sustained response to IFN therapy (p=0.009) in HIV positive patients compared to patients without HIV coinfection.
within each quasispecies, clones that replicate efficiently may become predominant.

New immunological selection is impaired, resulting in faster progression of HCV in some cases.

2.4.6 The impact of HCV on HIV

The impact of HCV on HIV disease is less clear, and study results are conflicting. However, a majority of research reports indicates that HCV does not increase HIV viral load or directly accelerate HIV disease progression. Among a cohort of nearly 900 HIV/HCV coinfected people, those with both viruses were not more likely to experience accelerated HIV disease progression, develop an AIDS-defining illness, or die from AIDS. HCV coinfection did not appear to reduce the effectiveness of HAART, and the researchers concluded that HCV should not be seen as a barrier to HIV treatment. Notably, in this cohort, coinfected people were less likely than those with HIV alone to be taking HAART and most deaths occurred in the untreated subjects.

One report showed that HIV/HCV coinfection appeared to have no effect on the progression of HIV disease or survival in 100 coinfected people treated between January 1992 and May 1997. It was concluded from that study there was “absolutely no difference” in HIV disease progression and survival as assessed by time from HIV diagnosis to AIDS diagnosis, from HIV diagnosis to death, or from AIDS diagnosis to death in HIV/HCV coinfected people compared with those who had HIV alone.

On the other hand, some studies suggest that infection with certain HCV genotypes may be associated with more rapid progression to AIDS or death. HCV genotype 1 was reported to be more common in people with HIV/HCV coinfec

(about 83%) than in people with HCV alone (about 70%), which may contribute to
more aggressive hepatitis C. Coinfection with HCV (but not HBV) was reported to be associated with increased risk of progression to AIDS defining illness and death. In addition, coinfectected people in this study experienced “consistently reduced recovery” of CD4 cells when treated to HAART compared with those who had HIV alone.

Several other studies confirm that immune recovery after starting HAART may be impaired in HIV/HCV coinfectected people. For example, in the November 25, 2000 issue of the lancet Gilbert Grenb, MD, and colleagues with the Swiss HIV cohort study reported in response to HIV treatment in 3,111 HIV positive participants, 1,157 of whom also had HCV. Both the coinfectected participants and those with HIV alone were equally likely to achieve HIV viral loads below 400 copies/ml after starting HIV treatment. But the coinfectected people were less likely than those with HIV alone to experience a CD4 cell count increase of at least 50 cells/mm³ (75% Vs 84%). By the end of follow-up about 8% of the co infectected participants had decreased OI compared to about 5% of those with HIV alone, and the death rate due to all causes was more than twice as high among the coinfectected participants.

More recent studies offer similar results related to immune recovery. At the 8th Retrovirus conference in Feb 2001, J. Martin and colleagues from Madrid reported that among 902 study participants with HIV 72% of whom were coinfectected with HCV responses to HAART differed dramatically. Participants with HIV alone experienced an average HIV viral load decrease of over 5,700 copies/ml and an average CD4 cell count increase of 111 cells/mm³. In contrast, the HIV/HCV coinfectected participants experienced an HIV viral load decrease of only 606 copies/ml and a CD4 cell count increase of just 53 cells/mm³. At the Barcelona AIDS conference Juan Antonio Pineda and colleagues from Seville, Spain,
presented evidence showing that CD4 cell recovery after starting HAART is slower in HIV/HCV coinfected people compared to those who have HIV alone. Likewise Maria Dorracci, MD, and colleagues from Rome also reported at the same conference that coinfected people had a poorer response to HAART.

The question of how HCV affects HIV disease remains unsettled. As HIV/HCV coinfected people live longer, more data will become available that should shed light on how HCV infection influences the long-term natural history of HIV disease.

2.4.7 Treatment of HCV coinfection in patients with HIV

Since 1992, several small IFN-α treatment studies in coinfected patients have been performed. The reasons for this restricted clinical approach were the common perception in many physicians that: 1) HCV is more difficult to treat in HIV-positive people; 2) toxicities will be greater in HIV positive people; and 3) HIV-positive people have a short life expectancy. Another problem arising in treating HCV-HIV coinfected people is that the potential for an overlapping toxicity caused by RBV, besides the interaction between RBV and pyrimidine analogues, has produced caution in using RBV in these patients. Now, the improved prognosis and life expectancy of HIV patients raise concerns for treating HCV coinfection.

As HCV-related liver disease progression is faster in HIV infected subjects, anti-HCV treatment should not be delayed without reason (active drug addiction, alcohol abuse, etc). The assessment of potential contraindications for antiviral treatment, compliance profile, and interactions with antiretroviral drugs is mandatory. Rates of sustained response to IFN monotherapy in HIV-HCV coinfected patients ranges from 0% to 20% (Bonacini et al., 2000).
Three uncontrolled pilot studies have assessed the efficacy and safety of IFN-α and RBV in combination in HIV-infected patients (Sauleda et al., 2001 and Landau et al., 2001). One study included 20 hemophiliacs treated for 12 months and showed a 40% rate of sustained response overlapping the rate observed in the mega trials on IFN and RBV in combination in HIV-uninfected patients (Sauleda et al., 2001). Two other pilot studies, performed respectively in 51 and in 17 HIV nonhemophiliacs seropositives, showed a 20% rate of sustained response (Landau et al., 2001). Dropout rates were heterogeneous ranging from 5% to 29%. HCV-RNA levels at baseline were significantly lower in sustained responders in the two larger studies (Sauleda et al., 2001 and Landau et al., 2001). Overall results reported in these studies seem to be slightly lower than those observed in HIV seronegatives. Even if larger studies are needed, differences in patients compliance, needs for dose adjustments and treatment duration or a less efficacy of RBV addition could have made the difference with HIV-uninfected subjects. The use of growth factor (granulocyte colony – stimulating factors and recombinant human erythropoietin) could probably maintain a larger proportion of patients on treatment with effective doses of both IFN and RBV. None of these studies identified a negative impact of the combination of IFN and RBV on HIV infection and efficacy and tolerability of concurrent antiretroviral therapy.

Results of treatment with IFN and RBV in patients without sustained response to IFN monotherapy confirmed the influence of the response to the previous cycle of IFN on efficacy of IFN and RBV. The sustained response rate was 42% in relapers and 15% in nonresponders to IFN monotherapy (Perez Olmeda et al., 1999). A retrospective, multicenter French study evaluated 21 previous IFN nonresponders and one relapser (Zylberberg et al., 2000). The sustained virological response (SVR) rate was three of 21. In this study, three patients had greater than
0.5-log copies/ml increase in HIV-RNA levels, including two who were undetectable before therapy. The authors did not discern the cause of the increase of HIV viremia. In one case, the HIV-RNA level decreased from 8000 to 1800 copies/ml after discontinuation of RBV, suggesting that a RBV drug interaction might be the cause of the increase of viremia (Zylberberg et al., 2000).

Regarding this argument, a brief correspondence provided further information about the effect of 6 months of IFN and RBV therapy on HIV-RNA levels in 12 patients receiving antiretroviral regimens including one or two of the pyrimidine analogues zidovudine, stavudine, and lamivudine (Morsica et al., 1997). Overall, there was a trend towards a reduction in HIV-RNA levels on therapy for HCV, although this may have depended on an early response to a recent introduction of HAART in some cases. Two subjects had a small, 0.3-log copies/ml increase in HIV-RNA levels, whereas another experienced a dramatic rise in HIV viremia after stopping antiretroviral medications. One half of patients experienced a drop of the absolute CD4 count of 10-25%. Eleven of 12 subjects had undetectable HCV-RNA at 3 months of therapy, but three of them experienced an HCV breakthrough at 6 months (Morsica et al., 1997). In conclusion, even if most of the studies did not show a clear negative impact of RBV and IFN in combination on HIV disease progression and antiretroviral treatment, strict monitoring of CD4 and HIV-RNA should be warranted to all patients taking these two drugs.

2.4.7.1 Timing of treatment for HCV

Antiretroviral therapy, particularly the protease inhibitor ritonavir, is associated with an increased incidence of hepatotoxicity in patients with hepatitis C coinfection (Sulkowski et al., 2000), which may impair the immune reconstitution after the initiation of HAART (Greub et al., 2000). Therefore, coinfected patients
with early HIV, such as a CD4 cell count of 350 cells/ml or more and a HIV-RNA level less than 30,000 copies/ml (b-DNA) or 50,000 copies/ml (RT-PCR), might benefit from treatment for hepatitis C before initiation of HAART (Bruno et al., 2000 and Nunez et al., 2001). On the other hand, patients with more advanced HIV should be placed on antiretroviral regimen before starting treatment for hepatitis C.

2.4.7.2 Special challenges in coinfected patients

There are many selected categories of patients who pose particular challenges to clinicians treating HCV infection.

Non-responders

Waiting for new available therapies, many physicians are considering long term maintenance therapy with IFN for patients not achieving a sustained response to initial treatment.

1. Improve hepatic histology.
2. Delay progression to cirrhosis.
3. Decrease the risk of HCC.
4. Avoid the need for liver transplant.
5. Improve life expectancy.

The decision to continue IFN in nonresponders must be individualized and based on how well the patient did tolerate the treatment, pre-treatment degree of inflammation and fibrosis, and the patient’s risk factor for disease progression.
24.7.2.1 Treatment of HIV-HCV coinfectected patients with cirrhosis

Although patients with compensated cirrhosis (Child-Pugh class A) have a lower rate of sustained response, they could be considered for treatment in absence of major contraindications. Potential expected benefits are a reduction of fibrosis, especially in those with "transition to cirrhosis" (stages 3-4) and a decreased risk of developing HCC (Nishiguchi et al., 2001).

24.7.2.2 HIV Patients With Extrahepatic Manifestations of HCV Infection

Extrahepatic manifestations, such as mixed cryoglobulinemia and monoclonal gammopathy, are frequently observed in HCV patients. Extrahepatic manifestations of HCV infection seem to be less frequent in HIV patients than in non-coinfected patients (Agnello et al., 2000). Symptomatic cryoglobulinemia was rarely if not observed in these patients, in contrast to patients with HCV alone. Lymphoproliferative disorders seem to have the same frequency than in a non-HIV population. HIV disease does not increase the frequency and severity of such manifestations. Probably the imbalance of the immune system, sustained by HIV, plays a role in reducing the number of HCV-related extrahepatic manifestations.

24.7.3 Optimal schedule and duration of therapy for HCV in patients with HIV

As mentioned above, the correct treatment schedule of immune-competent patients with HCV genotype 1 infection is generally 48 weeks of IFN and RBV, whereas 24 weeks of treatment is adequate for those with genotype 2 or 3. The optimal treatment duration for HCV has not yet been established in coinfectected
Although the HCV genotype is a known predictor of response to therapy in patients with HIV, there are no data to establish whether 24 weeks of treatment is sufficient for immune-suppressed patients with HCV genotype 2 or 3 infection. Large trials to determine the efficacy and safety of IFN plus RBV combination in HIV-infected subjects are in course. Therapy should be stopped if virological (negative HCV-RNA) response is not achieved after 6 months on combination therapy and sustained virological response is the only endpoint for treatment. If the treatment is administered with the aim to slow HCV disease progression, more treatment could be prolonged, possibly in the setting of controlled studies. The recommended dose of IFN for chronic hepatitis C in HIV-infected individuals is the same as that used in HIV-negative patients (three mega units thrice weekly). Regarding RBV, recent data have shown that doses of 800 mg per day may provide a similar efficacy—at least in patients with less than 65-75 kg of body weight—than using higher daily doses (Sauleda et al., 2003). This is a relevant aspect because RBV can induce severe dose-dependent anemia, mainly when used concomitantly with some antiretroviral agents. For those patients with severe intolerance or absolute contraindications to RBV (serious anemia, hemoglobinopathies, heart failure) monotherapy with IFN can be tried, especially in subjects infected by HCV genotype 2 or 3. The rate of sustained response with IFN alone is very low (15%), but it does not differ from that obtained in HIV-negative patients (Soriano et al., 1996).

2.4.7.4 General Recommendations for Treatment Management of HCV in patients with HIV

♦ Evaluate all HIV-HCV-infected patients for HCV therapy
♦ Consider the following in assessing candidates for treatment and deciding how aggressively to treat.
• Laboratory and histological findings.
• Severity and duration of liver disease
• Comorbidities
• Alcohol use
• Psychiatric disorders
• Life expectancy
• Vaccinate susceptible patients against hepatitis A and hepatitis B viruses, Pneumococcus, and Influenza virus
• Individualize duration of therapy according to patients and HCV viral characteristics
• Evaluate response to treatment at 6 months and consider long-term treatment in nonresponders with advanced liver disease.

2.4.8 New treatment strategies

Pegylated IFN is an alternative to IFN and RBV that seems to provide similar treatment efficacy without RBV side effects (Shiffman et al., 1999 and Zeuzem et al., 2000). The combination of pegylated IFN and RBV will further increase SVR (Manns et al., 2000 and Sulkowski et al., 2000) The more rapidly progressive course of HCV in individuals with HIV raises the question of whether there is a role for IFN maintenance therapy in coinfected non-responder patients. A maintenance approach produced histological benefit in non-responders with hepatitis C alone (Shiffman et al., 1999) as well as a pilot study of immunosuppressed HCV-infected liver transplant recipients showed a positive impact of IFN therapy on liver histology compared to absence of treatment. Pegylated IFN is a very promising maintenance agent because it achieves sustained drug levels with once weekly dosing. Studies of the impact of maintenance IFN therapy on liver histology are needed in coinfected patients.
24.9 Vaccination

Vaccination against hepatitis A and hepatitis B is recommended in individuals with HIV, HCV, and in those with coinfection. Response to both vaccines is reduced in patients with HIV (Neilsen et al., 1997).

24.10 Liver transplantation

For patients with end-stage liver disease, transplantation may be the only option for survival. According to the American Council on Transplantation, more than 50,000 people benefit from organ transplantation every year. The United Network for organ sharing concurs and has always allowed HIV-Positive patients to add their names to its organ waiting list.

Some questions regarding clinical, social, ethical (a short number of organs available for transplantation in many countries) and indeed, political aspects will influence these decisions. Anecdotal reports have pointed out successful orthotopic liver transplants (OLTs) in a few subjects with HIV-HBV coinfection and Child-Pugh C cirrhosis, (Boyd et al., 2001) but in patients with hepatitis C the results are less encouraging. (Boyd et al., 2001 and Ronald et al., 2001). Reinfection of the graft, leading to rapid liver failure is the major obstacle, and prevention of HCV reinfection must be pursued. Transplant surgeons have recently begun to design clinical trials for treating viral end-stage liver disease in HIV-infected individuals. At this point, it is crucial to address the following issues: 1) Who are the best candidates for OLT?; 2) What is the survival rate of HIV-transplanted patients in comparison to that seen in those who did not go to transplantation or with HIV-negative individuals with Child-pugh C cirrhosis?; 3) What is the influence of OLT on the progression of HIV disease (viral load, CD4 count, clinical symptoms)?; and 4) What is the course of the graft mainly when antiretroviral therapy is used concomitantly?
HIV AND HEPATOTROPIC VIRUSES IN INDIA

Despite detailed reports documented worldwide in association with HBV, HCV and HIV coinfection, a few reports have been published regarding the coinfection. Sud et al., (2001) have studied the prevalence of HBV coinfection in HIV positive patients. Out of 80 adult HIV positive patients 56 were male and 24 were females. Among them 27 (33.8%) patients (23 male and 4 females) were found to have coinfection with HBV. Of these 6 (22.2%) were HBsAg positive 22 were anti-HBc antibody positive and only one was positive for HBV-DNA. Four patients were found to have replicating virus (Three HBsAg positive, One HBV-DNA positive) with normal transaminases and advanced HIV infection. HBV coinfection was significantly higher among males (p<0.05). The risk factors for acquiring infection was heterosexual exposure in all HBV positive patients except one.

According to Kumarsamy et al., (2002) seven (6%) out of 116 HIV positive patients were positive for HBV and 4 (4.8%) out of 43 HIV patients were positive for HCV. Fourteen percent of HBV infected and twenty five percent of HCV infected patients had received blood transfusion; fifty percent of HCV infected patients were injection drug users.

Mathur et al., (2002) highlighted the prevalence of HIV infection in HBsAg positive cases. They have analyzed a total of 9450 serum samples from patients suspected of liver diseases. Out of 9450, 557 serum samples were HBsAg (5.89%) positive. Out of 557 HBsAg positive serum samples 34 (6.1%)were positive for HIV antibodies. According to a study on the co-infection of HBV and HIV in patients with liver diseases, 154 serum samples out of 888 (46.75% patients presented with acute viral hepatitis 29.22% with chronic viral hepatitis, 12.98% with cirrhosis of liver and only 11.03% with hepatic encephalopathy) were found to be HBsAg positive. Out of 154, only 4 (2.9%) HBsAg positive patients were detected
to be coinfected with HIV and all the four were found to be suffering with chronic viral hepatitis (Kumar et al., 2003).

Tankhiwale et al., (2003) reported the seroprevalence of HCV and HBV in HIV infected patients. A total of 110 HIV positive patients were taken for their study. Out of 110, 89 patients gave history of heterosexual high risk behavior. Of these 23 (25.8%) and 5 (5.6%) were positive and HBV and HCV respectively. 34 (30.9%) and 8 (2.27%) out of 110 were positive for HBsAg and anti-HCV antibodies respectively. 4 patients out of 110 were infected with both HBV and HCV. They have concluded that incidence of co-infection rises with HIV diseases progression. There is a significant difference in the co-infection rate, between the symptomatic (stage IV) and asymptomatic group (stage II) of HIV infected patients.

In view of the limited data available on interplay of HIV and hepatotropic viruses among Indian population, there is an urgent need to conduct detailed studies in a multifaceted approach to document the problem of HIV/hepatotropic viruses infection pattern in Indian patients.