CHAPTER III

LITERATURE REVIEW

History

During the 1970s and 1980s, following the development of diagnostic tests for hepatitis B virus (HBV) and hepatitis A virus (HAV) (12,13), the majority of post-transfusion hepatitis (PTH) cases were shown neither to be caused by HBV nor by HAV. In this respect, the term non-A, non-B hepatitis (NANBH) was first used (14). Attempts to identify these NANBH agents were unsuccessful until in 1989, using molecular technologies, the NANBH was identified from infectious chimpanzees plasma and was named hepatitis C virus (HCV) (1). This discovery has led to the development of an immunoassay to detect circulating HCV antibody that shown that HCV was the predominant cause of transfusion-associated NANBH around the world as well as being a major cause of sporadic NANBH (2). Moreover, it revealed the association between HCV infection and the development of chronic hepatitis, cirrhosis and even hepatocellular carcinoma (3, 4).

Biology

Most of the understanding about the nature of HCV is derived from analysis of cloned cDNA of its genome and from comparative analysis of its nucleotide sequence with that of other known viruses. It has been shown that HCV is distantly related to the pestiviruses and to a lesser extent the flaviviruses. Firstly, although the nucleotide of the genome and the encoded polyprotein are little overall primary sequence identity, these homologies are colinear regions (15,16), which indicated a similar genetic organization. Secondly, the hydrophobicity profiles of the polyproteins are remarkably similar (15). Thirdly, there is substantial primary sequence homology

(about 50%) at the 5' leader upstream of the large open reading frame (ORF) between that of HCV and pestivirus (15,17). Taken together, HCV is now classified as the prototype of a third genus of the flaviviridae family along with the pestivirus and the flavivirus genus (16).

Structurally, HCV virion has not yet well characterized morphologically and biochemically due to the low titer of this virus in both infected host and in vitro culture system (16). However, early studies using the infectivity of HCV in chimpanzees as a model have been concluded that HCV is a lipid-enveloped virus with a diameter about 30-60 nm. (18,19) and has a buoyant density of between 1.09 and 1.11 g/ml in sucrose gradient (20). Recently, immunoelectron microscopic studies have identified a putative HCV virion of 55 to 65 nm in diameter with spikelike projections (21).

The HCV genome is a single-stranded positive sense RNA molecule containing approximately 9500 nucleotides (nt) with a single large open reading frame that spans almost the entire genome (1,15,16). Figure 1 illustrated the organization of HCV genome and the encoded polyprotein precursor (16). The large ORF encodes a polyprotein precursor from which individual viral proteins (both structural and non structural) are processed co-and post-translationally through the combined action of host and virus-specified proteases (22,23). Most of the information available on the viral proteins of HCV and putative function have been derived from analyses of cDNA expression systems (both in vitro and in vivo). The structural proteins of HCV appear to be processed from the N-terminal region of the polyprotein at least in part through the action of host signal peptidase cleaving after internal signal sequences within the polyprotein (22,24). The action results in the production of a basic, nucleocapsid (C) protein with size approximately 22 kD at the extreme N-terminus of the polyprotein, and is followed by two glycosylated proteins (E1, 31-35 kD and E2, 68-72 kD) that are presumed to be envelop glycoproteins (22,24).

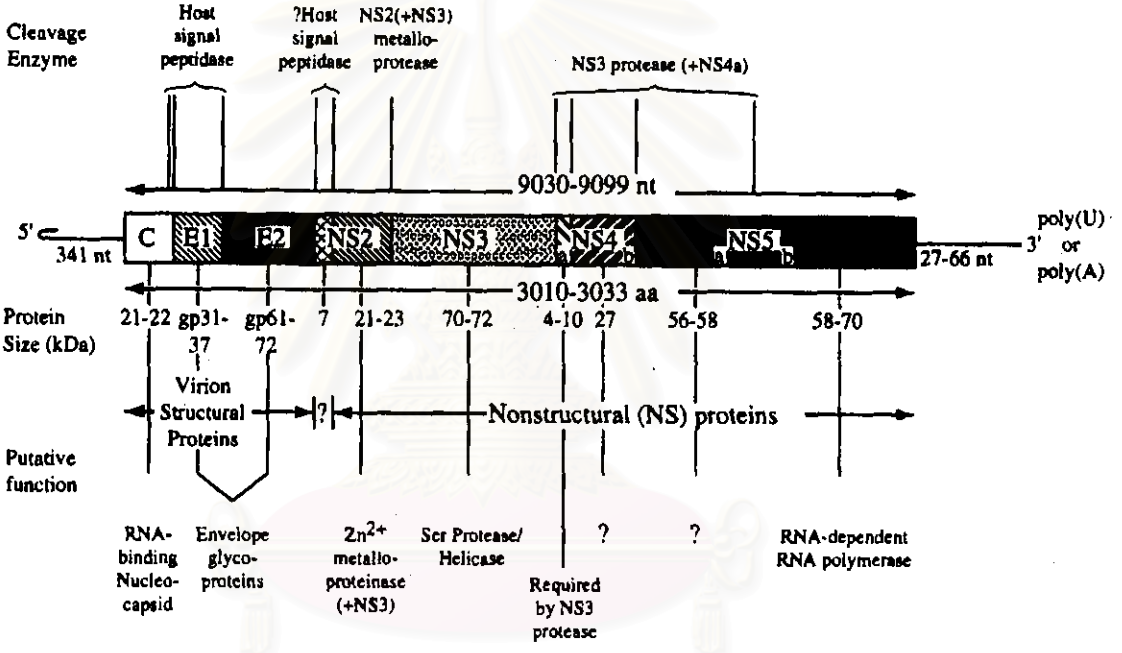

Figure 1. The genetic organization of the HCV genome and the encoded polyprotein precursors. (16)

The non-structural proteins of HCV appear to be processed from C-terminal region of the polyprotein in large part through the combined action of two virally encoded proteases, one within the ~ 23 kD NS2 protein and the other encoded within the 70 kD NS3 protein (25). This action results in the production of 6 presumed non-structural (NS) proteins (23,26). These consist of NS2 (~23 kD), NS3 (70-72 kD), NS4a (4-10 kD), NS4b (27 kD), NS5a (56-58 kD) and NS5b (68-70 kD). This nomencleature follows that used previously for the flaviviruses . The functions of some of the HCV NS proteins are known. The NS2 and NS3 proteins encode different proteases which are involved in the processing of the non-structural region (25). The NS3 protein also appears to encode a NTP binding helicase enzyme activity (15). The NS5b contains a sequence motif known to be highly conserved amongst RNA dependent RNA polymerases and thus likely to encode a similar function (15). The function of the other NS proteins are unknown, but recent studies have suggested that they are all playing a role in replication and assembly of the virus (15).

The HCV genome also contains 5' and 3' non-coding regions (NCR) that flank the large ORF. The 5' NCR of 341 nts precedes the initiator AUG codon of the polyprotein (17,26,27) and contains several short ORFs (28). The 5' NCR represents the most highly conserved sequence among different viral isolated (28), which suggests that it may play a very important regulatory role during viral replication, perhaps the initiation of translation since a small, hairpin, secondary structure could form at this 5' NCR. The 3' NCR has been identified downstream from the polyprotein, and shows significant variation, both in length and in sequence. It comprises 27 to 66 nts (15,17,26,27) followed by a presumed 3' terminal of poly U region in most isolated (26,27) whereas in strain of HCV-1, poly A region is seen (17).

Genetic heterogeneity of HCV

The HCV genome exhibits significant genetic heterogeneity, as a result of viral replication. Like other RNA viruses, HCV replicates through an error-prone RNA polymerase lacking proofreading activity, resulting in mutations. During persistent infection, HCV exhibits a high mutations rate at an average of 1.44-1.92x10^{6} base substitutions per site per year (29). At any one time, an infected individual may habour several closely related genomes containing a dominant sequence and a large spectrum of mutants, this genomic distribution referred to as "quasispecies" (30). In addition, different HCV isolated around the world also exhibit marked nucleotide sequence variability distributed throughout the viral genome when comparison of the complete and partial genomic sequence indicated that HCV is highly heterogeneous (15,31). This sequence heterogeneity is not evenly distributed over the genome but differs between regions. The 5' NCR is the most highly conserved region of the HCV genome (17,28), which is the usual locus of oligonucleotide primers used for detection of viral RNA by PCR-based methods. The putative core and NS3 region are also relatively well conserved (26) and antigens derived from this region are used in anti-HCV antibody assay. The hypervariable domains are located near the N-terminal part of the E2 envelop region. Sequential mutations in this region represent a mechanism by which HCV evades host immune surveillance and establishes and maintains persistent infection (32).

Variations in the genome allow HCV to be divided into distinct groups (1,15,33). Recently based on phylogenetic analysis of a 222 bp sequence within NS5b region, HCV isolates can be seperated into genotypes 1 through 6 containing subtypes a, b or c (33). Another study has described twelve genotypes based on E1 sequence analysis (34). It is likely that the genotypic classification of HCV may be clinically relevant. For example, the course and severity of disease may depend on the infecting genotype (35), Certain genotypes of HCV appear to be more responsive to interferon therapy than others (35). Finally, the extensive genetic heterogeneity of HCV has

important implications for future vaccine development (36) because it is likely that vaccine antigens from multiple genotypes will be necessary for global protection. In this connection, some genotypes of HCV show a broad worldwide distribution, whereas others are only found in specific geographic regions (33,34,37). For example, both blood donors and patients with chronic hepatitis from countries in Western Europe and the USA are commonly infected with genotypes 1a, 1b, 2a, 2b and 3a, although the distribution of each may vary (33,34,37). In Japan and Taiwan and probably parts of China, genotype 1b, 2a, and 2b are the most frequently found (37, 38). In Thailand, Singapore, Malasia and possible Bangladesh and eastern India, genotype 3 is found (37,39). In Egypt, the Middle East, and Central Africa, genotype 4 is the predominant found type (34, 37). Type 5 is the predominant genotype in South Africa (33, 37). Type 6 constitutes a significant proportion of HCV infections in Hong Kong (37) and Vietnam (40). Finally, type 7, 8 and 9 have been identified only in Vietnam (40). Overall, although many genotypes are widely distributed around the world, there are clearly dintinct differences in their distribution.

Clinical manifestations of HCV infection

Acute hepatitis C

The clinical features of acute hepatitis C are indistinguishable from other types of viral hepatitis. However, the incubation period for acute hepatitis C is usually intermediate between that for hepatitis A and B, with an average about 6 weeks, based on the study of transfusion recipients (41). Symptoms are usually mild or subclinical, and often milder than in either hepatitis A or B. Fatigue is the most common reported symptom, with only 25% of cases develop jaundice (14). The serum ALT levels are lower in patients with acute hepatitis C than those with acute hepatitis B. The elevated ALT levels occur in two differing patterns: monophasic, with a rapid decline, or multiphasic, with wide fluctuation. The multiphasic pattern may be associated with more severe disease or progression to chronic liver disease (42).

Fulminant hepatitis due to HCV infection seems to be very rare, and these rare cases are quite common in Japan than elsewhere (43). The association of HCV with fulminant hepatitis reported in Japan may reflect differences in the virulence of HCV genotypes, or the involvement of unrecognized cofactor. Acute HCV-HBV coinfection has been found in a number of cases of fulminant hepatitis (44).

Chronic hepatitis C

Approximately 50% of acute posttransfusion hepatitis C will develop chronic infection, about 20% of these individuals will progress to cirrhosis (3,45). The course of the disease is characterized by fluctuating and/or persistently elevated serum ALT levels more than 6 months (45, 46). Chronic HCV infection is often silent, progression to advance liver disease occurs without any symptom (47) until severe impairment of liver function is diagnosed. Male gender, old age, and a large viral dose have been found to influence the development of chronic HCV infection (48,49). Furthermore, transmission via transfusion seems to be associated with relatively aggressive forms of chronic hepatitis C (48,49).

Hepatocellular carcinoma is associated with HCV infection (4,46), usually occurring in patients with cirrhosis. In Japan, studies have indicated that HCV plays a major etiologic role in hepatocellular cancer (50), often following two or more decades of presumed HCV infection. Progression towards cirrhosis in HCV-infected individuals is probably enhanced in the presence of other risk factors such as co-infection with hepatitis B virus or human immuno-deficiency virus, or hepatotoxic agents such as alcohol (45,49).

There is a strong association between chronic HCV infection and autoimmune hepatitis (AIH) type-2 (51). AIH type-2 patients positive for anti-GOR autoantibody are also frequently positive for antibody to HCV (51). This anti-GOR antibody detects a human epitope sharing an amino acid sequence coded by the HCV core antigen gene. Anti-GOR is therefore presumed to be an autoantibody specifically

induced by HCV infection (51). The chronic HCV infection is also associated with mixcryoglobulinemia, vasculitis, cutaneous manifestation, and glomerulonephritis (52,53). Recently, the presence of thyroid autoantibodies, Hashimoto's thyroiditis, and hypothyroidism have been found associated with chronic hepatitis C in woman (54).

Sequence of HCV infection

The early stages of HCV infection are only partially understood. Figure 2 illustrated the sequence of transfusion-associated HCV infection. HCV RNA is the earliest detectable marker for HCV infection. Specifically, it has shown that in patients with PTH as well as in experimental infected chimpanzees, HCV RNA can be detected within 1 to 2 weeks of exposure (42,55,56). The mean interval between exposure to HCV and the first appearance of HCV RNA is usually very short. In contrast, a prolonged interval (up to 8 weeks) between the detection of HCV RNA and the onset of hepatitis (measured by elevated serum ALT levels), and an even longer interval (up to 12 weeks) before the first appearance of anti-HCV was also observed (42,55). During the serologically silent period, HCV RNA is the only marker that permits the diagnosis of primary HCV infection. Moreover, the detection of serum HCV RNA may provide important information on the outcome of the disease. HCV viremia is transient in acute self-limitting hepatitis, but it persists in the patients in whom acute hepatitis progress to chronic form, although intermittent viremia may seen in some cases of chronic hepatitis (55,56). The magnitude of this viremia has been difficult to quantitate, but estimates from 10^2 to 10^8 viral particles per ml of plasma in some studies (57). The presence of HCV RNA in serum is believed to be a sensitive and reliable indicator of infectivity (55), but the absence of HCV RNA does not ensure a noninfectious state (56).

Detection of antibody to C100-3 in ELISA-1 is typically positive several weeks after the onset of clinical hepatitis, although seroconvertion may be delayed up

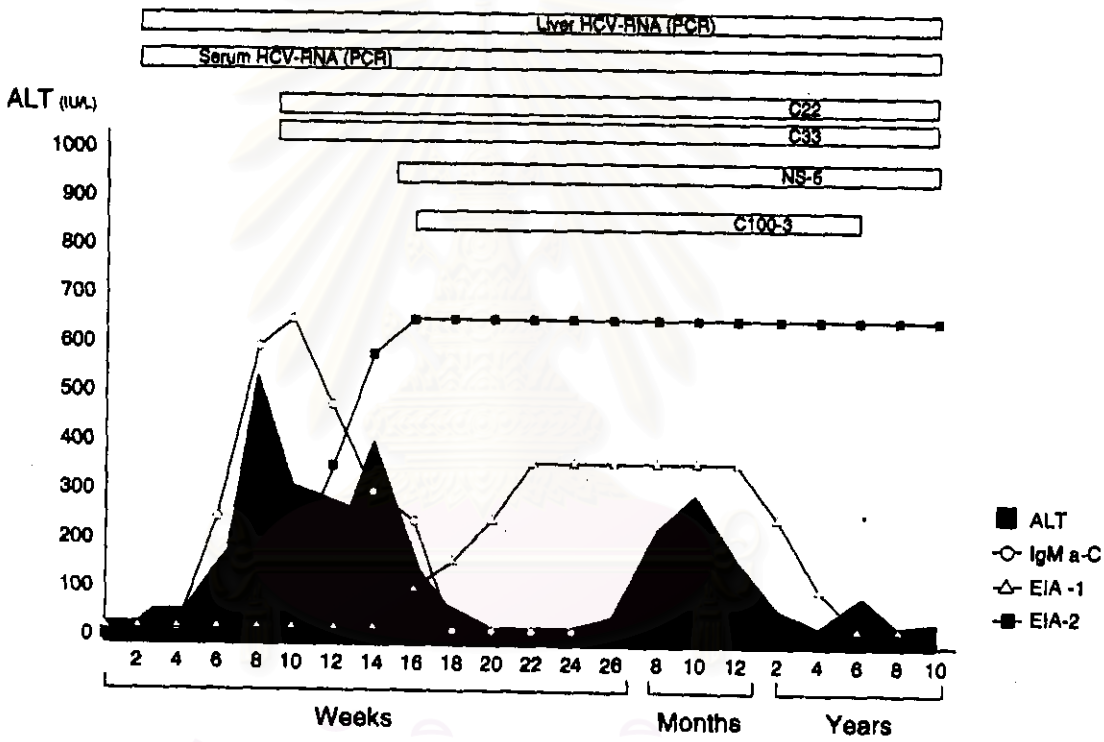

Figure 2. Representative sequence of events in transfusion-associated hepatitis C virus infection. (45)

to one year (42). In ELISA-2, detection of antibodies to the core (C22) and NS3 protein (C33) allow for earlier diagnosis of HCV infection (approximately 6 weeks) than the detection of antibodies to C100-3 (58,59,60). Antibodies to both C22 and C33 disappear in patients with resolving HCV infection but persist in patients with chronic hepatitis C (59). Persistance of antibody to C100-3 does not always correlate with persistance of infection.

Recent studies indicated that anti-HCV IgM was positive within 1 month of onset and was also detected for a long time beyond the acute phase of infection (45,61). IgM antibody to C100-3 was seen persistantly for over 6 months in patients that have chronic HCV infection, and was undetectable by 6 months in all patients with self-limitting HCV infection (45).

Changes in serum ALT levels have been reported in several studies of transfusion-associated hepatitis C (42,46,62,63). The ALT elevations tend to be segregated into two patterns. The monophasic pattern is one in which the ALT level rapidly reaches a zenith and then decreases soon thereafter. The other is a biphasic or multiphasic pattern in which lower ALT values are interspersed between two or more ALT peaks (46,63). Di Bisceglie, et al, reported the changes in ALT level during long-termed followed patients with chronic PTH (62). ALT level became elevated within 4-8 weeks, and reached its peak between 8-12 weeks after transfusion. It then declined progressively, with fluctuations, during the follow-up period (62). Although it has been suggested that the pattern of ALT elevation may have prognosis value in predicting chronicity (63), several studies have clearly showed that no ALT pattern can predict resolution of disease (46,62,64).

Epidemiology of HCV infection

The epidemiology of hepatitis C virus is remained unclear. Up to one-half of patients, the source of HCV infection is unknown and these cases are often described

as sporadic or community acquired (49). A further one-third of patient has a history of intraveneous drug abuse, while rather few have been exposed by blood transfusion, by sexually or other close human contact (49). Using first and second generation ELISAs for screening antibodies to HCV suggest rather high prevalence rate among blood donors: on the order of 0.2% to 0.5% in Northern Europe (65), and 0.5% to 1.5% in Southern Europe (65), the United States (65), and Japan (66). In Southeast Asia, the seropositive rates survey from Indonesia, Malasia, Thailand, Vietnam, and the Philippines are between 1.5% to 3.0% (66). In some parts of Africa, the prevalence rates of up to 10% have been reported (67). Since blood donors are preselected against risk factors of HCV infection, this is the lowest estimated disease prevalence in the general population. Thus, a very large number of people worldwide are infected with HCV. Within high risk group such as a multiple transfused hemophilic patients and intraveneous drug addicts, the prevalence of antibodies to HCV often exceeds 60-70% (42).

The main route of transmission is parenteral, and the majority of HCV infected individuals are intraveneous drug users or recipients of blood and blood products. In the early date, following the screening of blood donors for HBV, several studies indicated that HCV accounted for about 90% of PTH cases(41,68,69). HCV has also been transmitted by exposure to blood products. Most hemophilic patients who received clotting factors before the advent of viral inactivation procedures became infected with HCV (66,70). Hemodialysis patients are also at increased risk for contracting HCV, either with or without associated blood transfusion (65,66). Infection also can be transmitted by organ transplantation (71).

Intraveneous drug abusers are at greatly increased risk of HCV infection through shared needles and syringes (66). Tattoing and acupuncture have also been reported to be two potential transmission routes (66). Needlestick accidents in health care workers inoculated with blood-contaminated needles have led to HCV transmission, but the rate of infection is rather low (72).

HCV RNA has not been detected in semen, urine, stool, or vaginal secretions, and whether it is present in saliva remains controversial (73). Dentists and oral surgeons have a high risk of HCV infection than general population. Klein, et al, found that approximately 9% of dental surgeons and 1% of other dentists were anti-HCV positive (74).

Sexual transmission-Studies examining the risk of sexual transmission have ganerally been small and have yielded conflicting results. One study has found no evidence of HCV infection in spouses of patients (75). However, another study has indicated infection between spouses that have been confirmed by showing the close genetic relationship between viruses of each partner (76). There is also evidence showing a relationship between marriage time and spousal (76). This data has suggested that heterosexual transmission is possible but rare. The risk of household, nonsexual transmission and homosexual transmission of HCV is very low (66).

Transmission of HCV infection from mother to infant is uncommon and appears to be more likely if a mother is positive for both anti-HCV and HCV RNA than if she is positive for anti-HCV only. Approximately 10% of viremic mothers, mainly with high viral titers, have been shown to transmit infection to their babies (77). The risk of transmission may be much greater if the mother is co-infected with HIV (78). The presence of viral RNA in some neonatal serum born to HCV-infected mother raises the possibility of intrauterine transmission (78), but this does not seem to be a common event.

Laboratory diagnosis of HCV infection

Since there is neither in vitro system to isolate hepatitis C virus nor a serological assay to identify HCV antigen, the main diagnosis of HCV infection now are based on detection of specific antibodies to recombinant proteins or synthetic

peptides of HCV genome and detection of HCV RNA through gene amplification techniques, which will be summarized as follows:

1. Antibody screening tests.

The cloning and sequencing of HCV genome has led to the development of specific serological assays that has been used widely in blood bank and clinical settings for diagnosis of HCV infection. A prototype radioimmunoassay to detect circulating antibodies in NANBH was developed from the expression product of clone 5-1-1 for use in the original HCV research. Later, a variant of this clone (C100-3) was expressed in yeast to produce recombinant viral antigen in quantity enough for worldwide use (79). The first generation HCV antibody test was an enzyme-linked immunosorbent assay that detect antibodies directed against a recombinant fusion protein of human superoxide dimutase and a virally encoded nonstructural antigen of the NS4 region (so called C100-3 antigen) (figure 3) (2). The introduction of this test in screening of blood donors has been greatly reduced the risk of transfusion-transmitted HCV (68,69). Seropositive was high in patients with signs and symptoms or risk factor of HCV infection, both in transfusion settings and among sporadic cases (68,80). However, there were problems with false positive test, and the positive predictive value was poor when the test was applied to low risk population such as volunteer blood donors (68,69). In addition, the first generation ELISA had poor sensitivity, especially in acute HCV infection (2,81). This limitation led to the development of second and third generation ELISA tests (ELISA-2 and ELISA-3) (figure 3). The ELISA-2 includes additional recombinant antigens and detects antibodies to the core protein (C22-3 antigen) and the nonstructural protein NS3 (C33c or C200 antigen) as well as C100-3 antigen (82). The ELISA-3 includes another additional antigen from the NS5 region. These ELISAs have greater sensitivity and specificity than ELISA-1, since antibodies to C22-3 and C33c are usually detectable earlier, more frequently and longer period than C100-3 after HCV infection (58,59,60). Moreover, the core region of the genome is conserved between HCV types, which allow the higher rate of detection of antibody to C22-3 than

C100-3 (58). ELISA-2 and ELISA-3 are now widely used in blood donor screening to prevent transmission and development of posttransfusion hepatitis C in recipients of blood with 90-100% sensitivity (83). Although screening of blood donor with ELISA-2 and ELISA-3 has almost eliminated PTH-C, shortcomings to these assays exist. Anti-HCV antibodies may not occur until 3 months or as late as 6 months following infection (55,83). Moreover, immunosuppressed patients (eg. renal transplant recipients) occasionally have HCV infection without detectable antibodies and will only be detected with viral assays for HCV RNA (71). Furthermore, even with the high specificity (99.7%) of anti-HCV ELISA-3, false positive results are common among blood donors (84). The positive predictive value of the test depends on the prevalence in the donor population, which is very low.

2. Confirmatory tests or Supplemental tests.

For confirmation of positive anti-HCV ELISAs test results, supplemental assays have been developed. The most commonly used is recombinant immunoblot assays (RIBA, Chiron). Up to now , three third generations of RIBA tests have been used. In each generation of RIBA test, distinct HCV antigens using in each generation of the ELISA tests are applied seperately on nitrocellulose strips (figure 3), and after incubation with the patient's serum, the anti-HCV antibody recognition patterns are made visible. The RIBA generally allows determination of the specific antigens to which antibodies are reacting in the ELISA, thus may be helpful in identifying false positive ELISA results due to reactive with the superoxide dimutase. The first generation RIBA (RIBA-1) is no longer used because of its relative lack of sensitivity. In the third generation RIBA (RIBA-3), which seems to be the most specific assay so far, 5-1-1 is excluded, and two recombinant proteins (C22-3 and C100-3) are replaced by synthetic peptides. This modification improves the sensitivity of RIBA-3 over that of RIBA-2 (85). RIBA results are generally interpreted as positive when antibody to antigen of more than one HCV regions are detected. A high proportion (80-90%) of such HCV RIBA-3 positive individuals are viremic, as detected by PCR (86). However, among healthy blood donors, often with

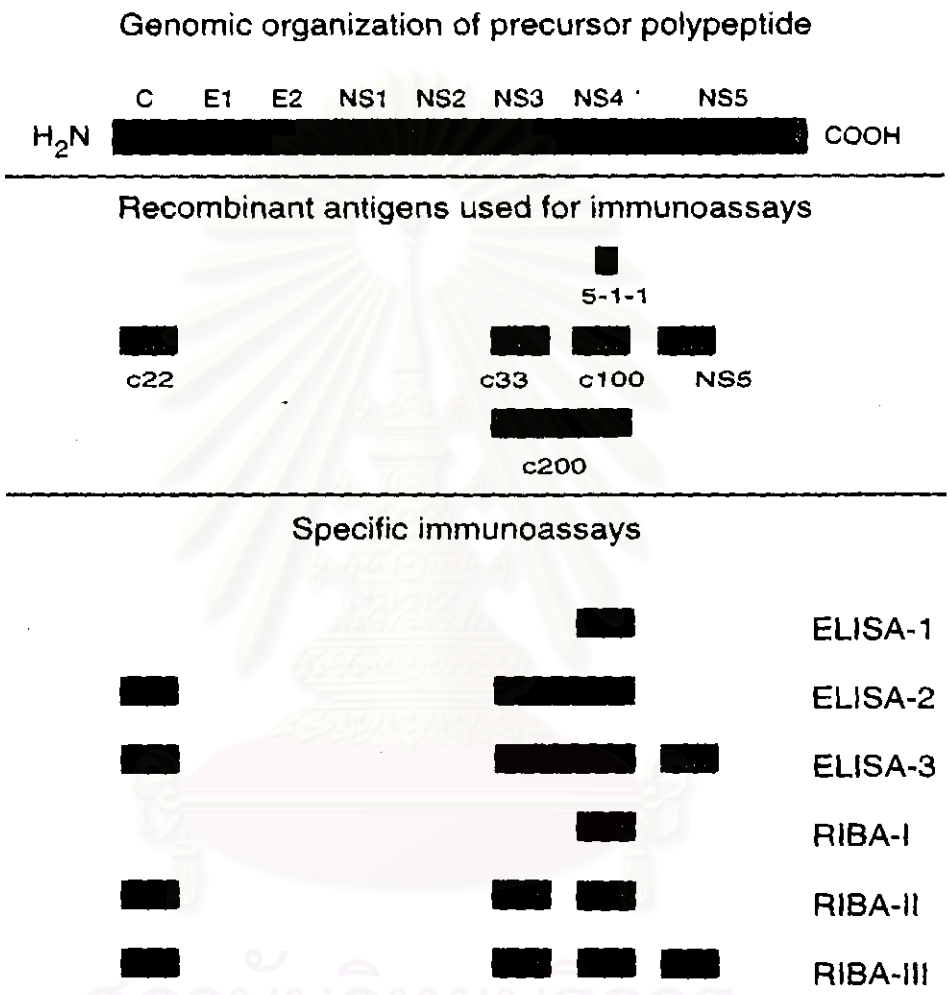

Figure 3. Hepatitis C virus polyprotein: recombinant antigens and immunoassays. (45)

RIBA-2, and to a lesser extent with RIBA-3, antibody recognition is found against one HCV antigen only (86). These patterns are interpreted indeterminantly, and may reflect non specific IgG binding, resolved HCV infection, or actual HCV viremia. During the initial period of acute HCV infection with increasing antibody response, there is occasionally weak or indeterminate RIBA, but also chronically HCV infected individuals may remain RIBA indeterminate over prolonged periods of time (87). Consequently, indeterminate RIBA test results should be reconfirmed by detection of HCV RNA. Anti-HCV recognition patterns in RIBA are of no prognostic value with regards to severity of disease, or possible clearance of HCV viremia (83).

3. Anti-HCV IgM assay.

Since currently available screening assays predominantly measure IgG antibodies which have been found to be inadequate for the early diagnosis of acute hepatitis C, methods for detection of IgM anti-HCV have been developed. Recently, several studies indicated that IgM antibodies to HCV core would be an earlier marker for acute and/or active HCV infection and could be detected in a substantial number of patients with chronic hepatitis C (60,88,89). In addition, the detection of anti-HCV core IgM appears to be associated with the presence of HCV viremia (88,89).

4. Detection of viral RNA.

In view of the limitations of anti-HCV testing, HCV infection can only be assessed by detection of the viral RNA. Currently, the most widely used method to detect circulating HCV RNA is PCR-based method (Nested Reverse Transcription/ Polymerase Chain Reaction or RT-PCR) (55,90). This method begins with an initial reverse transcription of viral RNA to create cDNA copy, followed by amplification of the viral cDNA during 2 rounds of PCR using nested sets of HCV-specific oligonucleotide primers. In order to achieve the optimal sensitivity, these primers usually represent within the relatively well conserved 5' non-coding region of the viral genome (17,28,91). This method is extremely sensitive and allows detection of minute quantities of HCV RNA even in plasma pool (90). However, it is important to

note that improper handling, storage, and/or contamination of serum or plasma samples can seriously endanger the results obtained using RT-PCR assays for viral RNA (92), and in a recent survey, only 16% (5 of 31) of participating laboratories performed faultlessly in assaying a coded panel of samples by RT-PCR (93). There are many advantages in detecting HCV RNA. The diagnosis of HCV infection can be established in early acute infection prior to antibody detection or aminotransferase elevation (42,55), as well as among patients who fail to develop antibody during the course of chronic disease (55). Dose and duration of antiviral therapy can be accurately adjusted when monitoring serum HCV RNA levels (57,94). Finally, the documentation of perinatal transmission of HCV is established with the presence of HCV RNA in the newborn blood (78). PCR can be used to detect HCV RNA not only in serum, but also in liver biopsy material (95). A negative PCR for HCV RNA in the serum correlates with an absence of HCV RNA in liver tissue in most cases (95). PCR is not very well suited for quantitation of the virus load in the infected individual, which was shown to be of importance in monitoring anti-viral therapy. Also a high viral load correlates with more severe histological abnormalities in liver biopsy material, and transmission of HCV from mother to infant is correlated to maternal viral load (96).

Another method developed recently to detect HCV RNA in serum is branched DNA assay. This assay involves the capture of viral RNA using immobilized DNA oligomers complementary to the 5' genomic RNA region, followed by signal amplification using branched DNA (bDNA) chemiluminescent detection probe (97). Detection of HCV RNA by bDNA assay is rapid, convenient, and gives consistently reproducible results in quantitating the virus. However, the bDNA assay is about 3 logs less sensitivity than RT-PCR (97), concentrations of HCV RNA less than $3.5x10^5$ geq/ml are undetectable. Recently, a commercially available HCV RNA assay, with a single enzyme for reverse transcription and DNA polymerization, has potential as diagnostic test (98).

Prevention of HCV infection

Since blood transfusion has been documented as a major transmission route of HCV infection, elimination of anti-HCV positive blood is important in preventing PTH. Current screening of blood donors for anti-HCV with second and third generation assays has virtually eliminated posttransfusion hepatitis C (83). Plasma products that have undergone heat treatment and/or chemical inactivation are considered safe (99) and has lessened the risk of HCV infection associated with the administration of these materials. The screening of kidney and pancreas donors for circulating anti-HCV also has minimized liver disease in these organ recipients (71) and measures for prevention of HCV transmission in dialysis units appear to be appropriate. Lastly, enhanced blood donor selection and needle exchange programmes may add to the prevention of further spread. The prevention of HCV infection transmitted through other modes awaits the development of vaccines and protective antibody preparations. The recent finding that chimpanzees were protected against a homologous viral challenge following immunization with recombinant DNA-derived envelope glycoproteins of HCV (36) offers substantial encouragement and optimism for achieving effective control of HCV infection. The efficacy of immunoglobulin preparation in preventing disease progression postexposure also remains an important area of future investigation, since an initial study in chimpanzees has shown that administration of gammaglobulins containing anti-HCV shortly after viral challenge delayed the onset of acute hepatitis (100).

Treatment of HCV infection.

Since the majority of HCV-infected patients will develop chronic liver disease, the most important of the treatment are to eradicate HCV and prevent on-going hepatic injury. Currently, Interferon alpha (IFN-α), at doses approximating 3M units three times weekly for the period of 6 months or longer is the most successful treatment for chronic hepatitis C infection in several studies (101,102). This treatment

results in significant reductions in aminotransferase abnormalities, associated with improvement in liver histology, approximately 50% of patients. In addition, these results also correlate with the reductions in HCV viremia level (57,94). Unfortunately, after therapy is stopped, about 70% of responders relapse with a return of serum ALT levels to the pretreatment range or higher (103). Therefore, overall, a sustained response (with normal ALT or HCV RNA still negative 6 months after therapy is stopped) occurs in only 10-30% of patients (103). Return of viremia in the absence of biochemical relapse also has been documented in some of these responders after therapy is stopped. Shorter duration of HCV infection, younger age, absence of cirrhosis, low serum HCV RNA level and presence of HCV type other than 1b tend to predict a positive response to interferon (83).

Interferon beta (IFN-β) also has been used to treat patients with chronic hepatitis C. Moreover, using this type of interferon treat acute hepatitis patients in one study, indicated that such therapy may reduce the incidence of chronicity (104). Patients treated with interferon may experience troublesome side-effect. Early common side effect is a flu-like which usually disappears within 3 weeks. Other side-effects include fatigue myalgias, leucopenia and thrombocytopenia; psychiatric syndromes often with depression; and autoimmune phenomena resulting in arthritis, thyroiditis, or hemolytic anemia may occasionally develop (49,83).