Clinical virology of and diagnostic approaches to HBV infection are sufficiently defined both in cases of acute hepatitis and chronic hepatitis with persistently active viral replication. However, many patients with chronic HBV infection may show an apparently suppressed viral activity. This is the condition of most HBsAg/anti-HBe positive individuals that can be either inactive HBsAg carriers with persistent inhibition of viral activity or subjects with episodes of virological/clinical reactivation alternating with phases of quiescent infection. There is still no reliable diagnostic tool for distinguishing between these two categories of patients during the stages of HBV inactivity. Another particular virological condition, usually related to a very strong suppression of HBV replication and gene expression, is the so-called occult HBV infection which is characterized by a persistence of viral genomes into the liver of HBsAg negative individuals. Many basic aspects and the possible clinical relevance of this kind of infection need to be further investigated. Finally, chronic HBV infection may also occur in patients coinfect ed with hepatitis C (HCV) or Delta (HDV) viruses. While the interactions between HBV and HDV are sufficiently understood, the interplays between HBV and HCV have not yet been completely defined.

Hepatitis B virus (HBV) infection may be associated with a very large spectrum of clinical forms that may occur in subjects of any age and with different immunological status. The data reported in this paper concern clinical/virological aspects of HBV infection in adult immunocompetent individuals.

Acute hepatitis

Acute type B hepatitis is usually a benign disease showing spontaneous resolution and recovery in more than 90% of cases. Very infrequently it may evolve into chronic hepatitis, while in 0.1-1% of the patients the acute hepatitis may have a fulminant course [1]. Its sero-virological profile is well defined: when clinical symptoms develop, the serum is positive for the virus "surface" and "e" antigens (HBsAg and HBeAg, respectively), there are high levels of IgM antibodies to viral core antigen (IgM anti-HBc), and the HBV-DNA is usually detectable by direct hybridization techniques [1]. When the virological/clinical course is self-limited, the viremia quickly decreases to undetectable levels, the HBeAg disappears in a few weeks, being replaced by the corresponding antibody (anti-HBe), while the HBsAg duration can be highly variable and usually becomes negative in 2-4 months [1]. The appearance of the antibodies to HBsAg (anti-HBs) is the best serological indicator of recovery from the infection, but in many cases it occurs several months after HBsAg seroclearance. A longer persistence of high HBV-DNA levels and of HBeAg positivity is considered a prognostic index of chronic evolution of the infection [2]: such an unfavourable outcome is conventionally diagnosed when HBsAg persists for more then 6 months from its first detection. It is of relevance to stress that in cases of fulminant acute hepatitis, because of the quick development of the massive hepatocellular necrosis, the clinical onset occurs when virus replication is already strongly suppressed, viremia decreased to very low levels, and HBsAg often not longer detectable [3,4]: in such cases detection of IgM anti-HBc is the most reliable assay for performing a correct etiologic diagnosis.

Chronic infection

Chronic HBV carriers may show different patterns in terms of viral serum markers and virus activity. Considering the viremia levels as an indicator of HBV replication, we may schematically distinguish these individuals in the following main categories: (A) HBsAg positive patients with persistently active viral
replication; (B) HBsAg positive individuals with permanent or temporary suppression of viral replication. Moreover, defining each circumstance of long-lasting persistence of viral genomes in the liver as chronic HBV infection, an additional category (C) may be considered that of the HBsAg negative subjects with occult HBV infection.

**HBsAg positive patients with persistently active viral replication**

The vast majority of HBeAg positive patients and a minority of the anti-HBe positive subjects belong to the category of patients showing a constantly active HBV replication. These cases show levels of circulating viruses >105 copies/ml and can easily be recognized by searching for serum HBV-DNA by direct hybridization techniques (figure 1). In these cases, the analysis of liver DNA extracts by Southern blot should reveal a high amount of HBV replicative intermediate forms. However, it has to be stressed that evaluation of intrahepatic HBV genomes in HBsAg positive patients makes sense only for research purposes, while it appears to be useless from the clinical point of view since it does not provide additional information to that obtained by the analysis of the serum.

**HBsAg positive individuals with permanent or temporary suppression of viral replication**

The category of chronic HBsAg positive patients with long-lasting or intermittent inhibition of HBV replication is highly heterogeneous and comprises subjects coinfected by hepatitis Delta virus (HDV), by hepatitis C virus (HCV), the so called inactive HBsAg carriers, and individuals showing episodes of virological and clinical reactivation occurring in the course of an apparently quiescent infection.

- HDV is one of the strongest inhibitor of HBV activity known so far [5]. The vast majority of HDV coinfection patients are anti-HBe positive and show viremia levels even lower than 103 copies/ml and, consequently, detectable only by the most sensitive polymerase chain reaction (PCR) amplification techniques [6]. In a few cases, however, HBV maintains high levels of replication [7]: it is unknown why HDV does not exert its usual suppressive activity in such cases.

- HBV and HCV shares modes of transmission, and their combined infection is a quite frequent occurrence, particularly in areas where the two viruses are endemic and among subjects with a high risk of parenteral infections [8-12]. Moreover, much evidence suggests that dual infection by HBV and HCV is of clinical relevance since it may be associated with severe forms of chronic liver disease poorly sensitive to Interferon treatment and with a high risk of hepatocellular carcinoma (HCC) development [13-16]. Despite these considerations, the virological profiles of HBV and HCV and their interplay in cases of coinfection is still largely undefined, and this is because most of the studies on the natural and post-therapeutic course of chronic hepatitis C have, among the main exclusion criteria, the coexisting presence of HBsAg and, vice versa, anti-HCV positive subjects are excluded from the studies on HBV-related liver disease. From the virological point of view, these patients may show different profiles: they may be either HBeAg or anti-HBe positive; in many of them HBV viremia levels are reported to be very low with high levels of HCV-RNA, but in some cases the HBV copy number per ml of serum is >105 while HCV viremia is very low; analogously, the replication activity of both viruses appears to be enhanced in some cases and suppressed in others [17,18]. However, all these data come from cross-sectional studies and, consequently, we do not know whether the activity revealed for each of the two viruses is the expression of a long-lasting state or is only a temporary effect within a complex kinetics evolving over years of chronic infection. This point has a great relevance in terms of both correct diagnosis and therapeutic approaches in cases of HBV and HCV coinfection, and it could be solved only if a proper number of such patients are longitudinally studied by testing serum samples serially collected during a long follow-up period without interfering treatments.

- Inactive HBsAg carriers – previously defined healthy HBsAg carriers – are a large part of the HBV infected individuals worldwide. They have normal liver biochemistry and ultrasonography, and if a liver biopsy is performed the histology is normal or reveals minimal changes. From the virological point of view, all these subjects are anti-HBe positive and persistently IgM anti-HBc negative, while the few data
at present available regarding their viremia levels suggest an amount of circulating viruses comprised between 10^3 and 10^5 copies/ml in most cases [6,19]. Further studies are needed to verify whether these values are stable over time, while the definition of the most suitable assay for the correct and early identification of these individuals takes high priority.

Several HBsAg carriers show a peculiar virological and clinical picture characterized by prolonged phases of inhibited HBV replication and apparent quiescence of the liver disease alternating with phases of reactivation of the viral replication leading to the exacerbation of the liver damage [reviewed in 20]. The early identification among HBsAg carriers of patients with such kind of HBV behaviour is of great clinical relevance since the episodes of reactivation may have a fulminant course [21] and because these patients are at high risk of developing cirrhosis and hepatocellular carcinoma [20]. Some of these individuals are HBeAg positive, but in some geographic areas like the Mediterranean basin this subgroup of patients is typically related with the anti-HBe positive state [21-23]. During the phase of active infection the diagnosis can be easily formulated because of the high viremia levels and the clearly positive values of IgM anti-HBc [23]. On the contrary, these forms are very difficult to identify during the phases of silent infection when the viremia levels appear to be identical to that observed in HBsAg carriers with permanently inactive viral replication (from 10^3 to 10^5 copies/ml) (figure 2). Some reports suggest that the use of a quantitative IgM anti-HBc assay may help in distinguishing between true inactive HBsAg carriers and anti-HBe positive patients with only transient remission of HBV activity [25]: this observation is potentially of great relevance and needs additional and more extensive studies to be definitively confirmed.

Summarizing, HBsAg positive individuals found to be anti-HBe positive with normal liver function tests and viremia levels <10^5 copies/ml are difficult to classify and they must be properly followed up over the years. Longitudinal studies involving large series of these carriers are necessary to define the times of the follow up and the most useful assays for performing correct diagnoses.

Occult HBV infection

Many studies performed in the last two decades have clearly demonstrated that HBV infection may persist also in the absence of circulating HBsAg [reviewed in 26-29]. This occult infection mostly associates with individuals positive for antibodies to HBV antigens (anti-HBc ± anti-HBs), but it also occurs in a considerable number of individuals negative for all serum markers of HBV infection [30]. Moreover, it is now evident that these patients with occult infection may carry both integrated and free episomal forms of HBV-DNA, in analogy to subjects belonging to the HBsAg positive categories considered above [31]. In some cases, lack of HBsAg detection is due to rearrangements in the HBV genome that interfere with gene expression or lead to the production of an antigenically modified S protein that is not recognized by commercially available kits [32-34]. However, in most cases HBV has no relevant genomic modification and the occult infection is a consequence of a profound suppression of viral replication and gene expression [30,35,36]. The molecular/immunological mechanisms involved in this inhibition are at present largely undefined.

The essential question in occult HBV infection issue is whether or not it has any clinical impact. In this connection, its potential role in HBV transmission and as a risk factor for HCC development has been sufficiently documented, although many aspects in terms of pathogenic mechanisms and frequency of occurrence still need to be clarified. Undoubtedly, carriers of occult infection may transmit HBV in the event of blood transfusion or organ transplantation and the recipients may develop a type B hepatitis that may have all the possible outcomes of the typical HBV infection, including the fulminant course and the development of a chronic hepatitis [reviewed in 26-29]. Moreover, many epidemiological and molecular studies performed since the '80s indicate that a persisting HBV infection might be involved in the development of HCC also in HBsAg-negative patients [reviewed in 26,28,37]. This evidence is confirmed by data in animal models showing that both woodchucks and ground squirrels, once infected by the corresponding hepadnaviruses (WHV, GSHV), are at high risk of developing HCC also after the apparent clearance of the virus [37,38]. Occult HBV should contribute to hepatocellular transformation through the same mechanisms traditionally considered the basis of the tumorigenic properties of the HBV [37].
Therefore, occult HBV infection appears to have relevance in terms of infectivity and contribution to HCC development, but whether it may induce liver injury is still unsolved.

Several reports showed that HBV genomes may persist for decades after recovery from self-limited acute hepatitis [40,41], suggesting that the occult infection is inoffensive in itself and it might become injurious only in the event of viral reactivation occurring under immunosuppressive circumstances [42]. Nevertheless, there is growing evidence that HBsAg sero-clearance does not necessarily imply a good prognosis [43]. Moreover it has been shown that patients with either idiopathic or HCV-related chronic liver disease have a high prevalence of occult HBV infection: among HCV patients, those carrying the occult HBV appear to have a more severe and precocious liver disease and a poorer response to Interferon-α therapy [30]. All these data provide further support to the hypothesis that occult HBV infection might be responsible for liver injury, although much more research is needed to fully clarify the biological basis and clinical meaning of this peculiar kind of infection.

Viremia levels are very low in patients with occult HBV infection because of the above-mentioned strong suppression of the viral replication. In these cases HBV genomes may persist in a measurable amount in the liver, while in the serum they are often undetectable even by the very sensitive nested PCR amplification techniques [26-30] (table 1). Consequently, a reliable diagnosis of occult HBV infection can be performed at present only through the analysis of liver DNA extracts by amplification techniques.

In conclusion, despite the almost forty years that have passed since discovery of the hepatitis B virus, several clinical and virological aspects related to its infection are still incompletely understood. These gaps are partly due to the insufficient number of clinical and diagnostic trials that have been performed in the past ten-twelve years. It is to be hoped that in the near future basic and clinical scientists will cooperate in improving the knowledge of this virus, which is the most important cause of liver disease worldwide.

REFERENCES


36. Loriot M.A., Marcellin P., Bismuth E., Martinot-Peignoux M, Boyer N, Degott C, Erlinger S, Benhamou J-P. Demonstration of hepatitis B virus DNA by polymerase chain reaction in the serum and the liver after spontaneous or therapeutically induced HBeAg to anti-HBe or HBsAg to anti-HBs seroconversion in patients with chronic hepatitis B. Hepatology, 1992; 15: 32-36.


FIGURE LEGENDS

Figure 1: HBV DNA detection by direct hybridisation techniques.
A – In house dot blot hybridisation method for detection of serum HBV DNA. Black dots correspond to positive serum samples. In line F 1:2 serial dilution of cloned viral DNA.
B – Intrahepatic HBV DNA detected by Southern blot technique. Smears in lines 1,3, and 4 reveal the presence of virus replicative intermediates in the liver extracts from the corresponding cases.
In line 5 ran the Eco R1 digested ?-phage DNA as marker of molecular sizes. The autoradiographic band in line 6 marks the position of the 3.2 Kb linear HBV DNA.

**Figure 2:** Alanine-aminotransferase (ALT), IgM anti-HBc, and HBV DNA profiles in long lasting follow-up of two HBsAg/anti-HBe positive patients corresponding to (A) an inactive HBsAg carrier, and (B) an individual showing episodes of virological/clinical reactivations alternate to prolonged phases of apparently quiescent infection (this last case adapted from Raimondo et al, see reference 24)

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<tr>
<td>HBV DNA</td>
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Table 1 - HBV DNA detection in serum and liver samples from 250 HBsAg negative individuals with chronic liver disease (adapted from Cacciola et al, see reference 30)

Figure 1
Figure 2

A

ALT
UI/L

Months
0 3 6 9 12 15 18 21 24 30 36 42 48 54 60

HBc-IgM

HBV-DNA

B

ALT
UI/L

Months
0 3 6 9 12 15 18 21 24 27 48 51

HBc-IgM

HBV-DNA