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REVIEW

Pathomorphological Characteristics and Pathogenesis of Viral Hepatitis*

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Viral hepatitis (VH) is an inflammatory reaction of the liver to hepatotropic viruses. Acute VH can be classified according to the virus and type of necrosis. Chronic hepatitis (CH) might be active, persistent or lobular based on previous classification. More recently the grade (necroinflammatory activity) and stage (fibrosis and architectural distorsion) of CH have been distinguished and scored. Apoptosis and necrosis probably coexist in VH and contribute to hepatocyte death. Several "death factors", such as transforming growth factor β, Apo1/Fas and tumor necrosis factor play a role in the execution of cell death. Injury of hepatocytes during viral infection can occur as a direct effect of the virus or as a result of the host immune response. Expression of different viral antigens can be detected during VH and might be visualized. Phenotyping of the portal inflammatory cell infiltrate in CH has shown a T-cell zone comprised of $CD4^+$ helper T cells and $CD8^+$ supressor/cytotoxic T cells at the periphery of the lobules. The pathogenetic mechanisms responsible for the final outcome of viral infection depend on viral factors (such as genotype, mutation etc.), virus-host interaction, expression of viral protein, several cytokines etc. which finally lead to the well known histological alterations of viral hepatitis. (Pathology Oncology Research Vol 2, No3, 132–143, 1996)

Key words: pathomorphology; pathogenesis; viral; hepatitis

Histological characteristics of viral hepatitis in relation to the activity and stage of the disease

Virus hepatitis (VH) is a diffuse inflammatory reaction of the liver caused by hepatotropic viruses, which besides the well-known types (A,B,C,D,E), seem to have new members.[64] Acute hepatitis (AH) is characterized by a combination of hepatocellular degeneration, necrosis and regeneration, inflammatory infiltration and macrophage activity[98] *(Fig.1)*. The proportion of these components may vary according to the particular virus, the host response, and the passage of time.

Several histological classifications of acute hepatitis exist.[98] The main event of AH is hepatocellular damage/necrosis, which can be *spotty (focal) necrosis*, when the individual hepatocytes (HC) die and are removed. The reaction is an immunocompetent elimination of cells carrying viral antigens and it is a hallmark of classic acute hepatitis. *Confluent and bridging necrosis* is the death of groups of adjacent HCs. Confluent necrosis linking vascular structures is known as *bridging necrosis*, which can be *"central-central"*, or a more severe form which links terminal hepatic venules to portal tracts *("central-portal")*. Confluent and bridging necrosis indicate a more extensive form of VH. Usually the more extensive the HC damage, the more florid the portal inflammation. *Piecemeal necrosis* is the death of HC at the interface of parenchyma and connective tissue with a variable degree of inflammation and fibrosis. *Interface hepatitis* is an alternative term introduced by Scheuer.[98] Piecemeal necrosis is the key feature of chronic active hepatitis (CAH). It is defined as a gradual destruction of a single or few hepatocytes at the border or interface of the mesenchymal, parenchymal margin and it is associated with inflammatory reaction. A very close contact between the cell membranes of HCs and lymphocytes, called peripolesis, has been pointed out[50] *(Fig.2)*. Lymphocytes destroy the periportal

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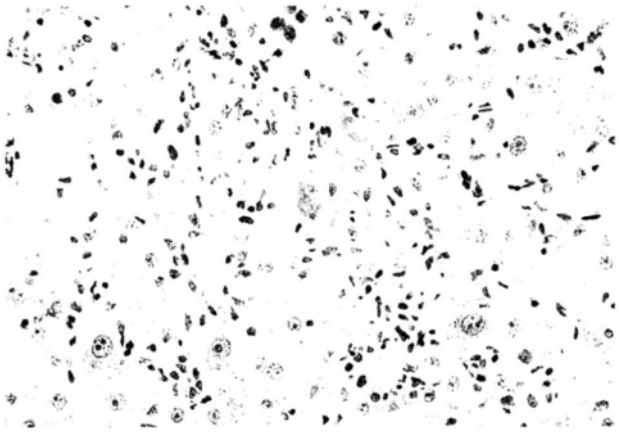

Figure 1. *Acute viral hepatitis (HE).*

parenchyma, the limiting plate is broken and lymphocytes replace the HCs.

It is generally accepted that it is not currently possible to differentiate reliably between the forms of acute VH caused by different hepatotropic viruses based on histology alone. However, there are certain characteristic histological features caused by the different viruses.[3,23,98,99,116] *(Table 1).*

Table 1. **Some characteristics of AVH caused by different viruses**

Hepatitis	*Histopathology*
AVH-A	dense portal/periportal inflammation and necrosis dominate, little perivenular necrosis, plasma cells prominent
AVH-B	peri/emperipolesis is prominent
AVH-C	bile duct damage, microvesicular fat, lymphoid follicles
AVH-D	eosinophilic change, microvesicular fat
AVH-E	similar to AVH-A

There have been many discussions of the nomenclature and classification of *chronic hepatitis* (CH)[6,7,8,63,67,104] after the definition in 1968[25] which recommended a simple division of CH into chronic persistent (CPH) *(Fig.3)*, chronic active (CAH) *(Fig.4)* and latter chronic lobular forms. The terms CAH/CPH are meaningful only if they are used in association with etiologic designations and are supplemented with additional quantitative information.[3,69]

The first numerical scoring system for measuring histological activity in CH was suggested by Knodell et al in 1981.[51] While widely used, it has recently been criticized. Recent editorials[26,46] discuss CH as a spectrum of common inflammatory reactions, the histological presentation of which oscillates in grade and may be modified by structural alterations such as fibrosis or cirrhosis, and may further have different prognostic and therapeutic implications according to etiology.[17,63]

More recently, the original histology activity index (HAI) has been divided into the *grade* and *stage* of the CH. *Grade* describes the intensity of necroinflammatory activity in CH, while *stage* measures the fibrosis and

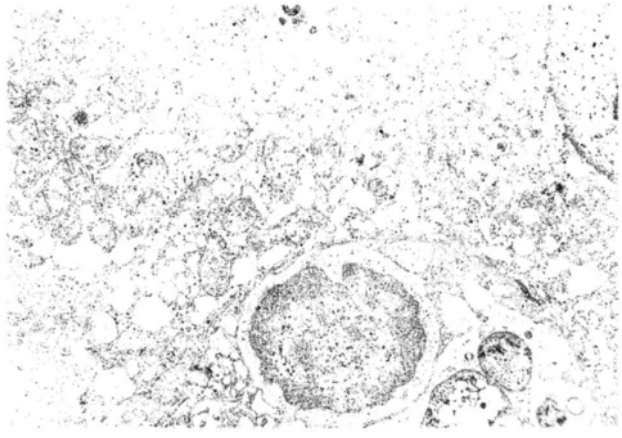

Figure 2. *Close contact of a lymphocyte and a hepatocyte in chronic hepatitis by EM (Councilman).*

Figure 3. *Chronic persistent hepatitis (HE).*

Figure 4. *Chronic active hepatitis (HE).*

architectural distortion of the liver.[26,46] Numerical scores are used to quantify both grading and staging providing a semiquantitative evaluation of the observed histological features. The modified HAI for grading includes (total score of 18):

- Periportal or periseptal interface hepatitis (piecemeal necrosis) (score: 0-4)
- Confluent necrosis (bridging, panacinar, multiacinar) (score: 0-6)
- Focal (spotty) lytic necrosis, apoptosis, focal inflammation (score: 0-4)
- Portal inflammation (score: 0-4)

Staging concerns fibrosis, architectural disturbances including cirrhosis, and scored from 0-6.[26,46]

The international group which has formulated the most recent semiquantitative scoring system underlines that "each pathologist is, however, free to use whatever system he or she wishes".[46] The scoring of liver biopsy is especially important in therapeutic trials.

The mode and mechanism of hepatotropic viral infection

It is now generally appreciated that both *apoptosis* and *necrosis* may coexist in human hepatitis and may contribute to liver cell death. Patel and Gores[86] point out that apoptosis has been "underrecognized" during liver injury because apoptosis is histologically inconspicuous in most liver alterations and the term necrosis is generally applied.[86] However, necrosis is correctly defined by loss of the plasma membrane permeability barrier, which defect results in cytolysis. *Apoptosis* is ("falling of") defined as nuclear DNA and cell fragmentation with preserved plasma membrane and organelle integrity. The cell fragments are ultimately eliminated by phagocytosis resulting in the histological appearance of cell drop out. Typical apoptotic cells can be observed in acute and chronic hepatitis as large Councilman-bodies *(Fig.5)*, or smaller, membrane-bound cell fragments, many of these already phagocytosed by neighboring cells and macrophages. It has been suggested that the elimination of virus-infected cells by apoptosis rather than by necrosis may have a significant advantage for the host. Apoptotic bodies will be phagocytosed while their membranes are still intact, which should prevent release of virions and other intracellular constituents and prevents tissue inflammation.[101,102] In contrast, lytic necrosis of cells might lead to viral dissemination.

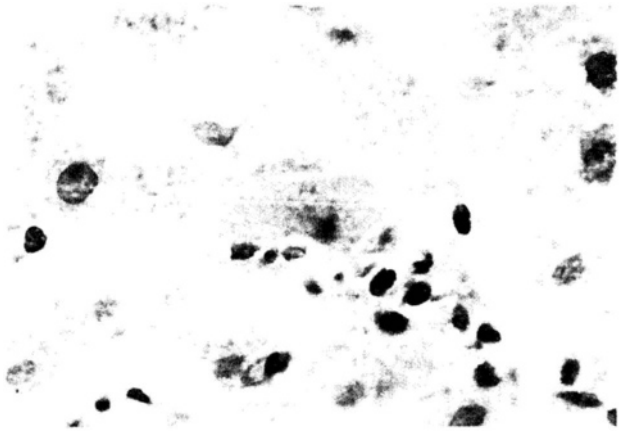

Figure 5. Apoptotic body in viral hepatitis (HE).

It has been suggested that hepatocytes progress from a putative progenitor area from the zone 1 (periportal) toward the perivenular region (zone III) where hepatocytes are eliminated by apoptosis ("streaming liver hypothesis").[2] The average duration of apoptosis in rat liver was found to be 3 hrs, which explains the relatively rare observation of apoptosis in histological sections even in states of considerable cell loss. The latter stages, as well as cell fragmentation and phagocytosis of apoptotic bodies, appear to be very rapid events lasting only a few minutes. A specific mechanism is involved in the recognition of apoptotic cells, which may vary with cell type.[86] By far the slowest part of morphologic features of apoptosis is the intracellular degradation of phagocytosed apoptotic bodies.[101]

The biochemical and molecular events in cells "destined" to die have been extensively studied and the genes and proteins involved in the active self-destruction of cells have been the focus of intensive research efforts.[101]

The "phases" of active cell death can be divided into a *preparatory* phase, during which events favoring or inhibiting active cell death will lead to a *point of no return*, which is followed by the *execution* of death.

Different *proteases* are very important in the *preparatory phase* in viral hepatitis. It is known that cytotoxic T-lymphocytes and natural killer cells inject a pore forming protein (perforin) and group of proteases (fragmentin) into the target cells. Perforin is integrated into the membrane. The proteases which have serine protease activity are responsible for the appearance of apoptotic changes in the nucleus of the target cell.[86]

Interleukin-1β (IL-1β)-converting enzyme (ICE) which shares functional and sequence homology with ced-3 (a gene in hematoides C. elegans responsible for apoptosis) causes apoptosis. Certain viral products can inactivate ICE and prevent ICE-mediated apoptosis and it has been suggested that inhibition of host cell apoptosis is a mechanism of viral survival.[86]

Tissue transglutaminases (TTG) catalyze the formation of glutamyl lysine cross-linkages between appropriate substrates, which result in the formation of a rigid, insoluble structure during the formation of an apoptotic body. The cross-linked protein may serve to trap cytoplasmic organelles[86] and prevent their release into the extracellular surrounding. TTG induction and activation occurs

during apoptosis in hepatocytes and it is not expressed in normal hepatocytes.[86]

Genes that control apoptosis are very important as a mechanism to counterbalance cell proliferation.[86] *Bcl-2* gene is a critical regulator of apoptosis and its product has been localized to mitochondria, nuclear membranes and endoplasmic reticulum.[43] Bcl-2 may play a role in the prevention of oxidative damage to cellular constituents, such as DNA, which may ultimately trigger apoptosis.[86] Several virus-encoded bcl-2 homologs have been identified (BHRF-1 in EBV, LMWS5-HL in African swine fever virus etc.) which have the potential of inhibiting apoptosis and they can contribute to viral latency or result in a persistent viral infection in the absence of cell lysis.[15,62] Other genes and their products such as p53, myc, TRPM-2 and RP8 are probably important in the regulation of apoptosis, however their function is not clear.

The "*execution* of cell death" is usually very rapid. The "death command" activates proteases, endonucleases, transglutaminase etc.

Positive and negative factors contribute to active cell death. *Negative* signals, survival factors, which prevent cells from suicide, are important for the maintenance of the appropriate tissue size.[101] In the liver, hepatotropic factors such as epithelial growth factor (EGF), hepatocyte growth factor (HGF), transforming growth factor α (TGFα) probably exert survival factor activity.[100,101]

The findings that the accumulation of hepatitis B surface antigen (HBsAg) in the cytoplasm of infected hepatocytes is associated with increased expression of a potent hepatocyte growth stimulator, the transforming growth factor-α (TGFα), suggests a possible pathomechanism for how a virus might stimulate cell proliferation.[73,96] Using double immunohistochemistry, both HBsAg and TGFα can be detected in the cytoplasm of the same hepatocytes. The increased production of TGFα can be proved by in situ hybridization (Schaff et al under publ.).

Positive signals which favor active cell death (death signal factors) participate in the selective elimination of damaged cells. These death signal factors include the TGFβ family, the Fas ligand and tumor necrosis factor (TNF).[101]

The effect of *TGF*β and related peptides have been studied extensively in the liver. The mitosuppressive action of TGFβ is well known. Schulte-Hermann's group has found that $TGF\beta_1$ induces apoptosis in cultured rat hepatocytes and in vivo when injected in rats some hours before sacrifice.[83] It has been suggested that $TGF\beta_1$ acts synergistically on cells already primed for apoptosis by unknown signals, which may be generated by mitogen pretreatment or by hyperplasia. It has been shown that hepatocytes, apparently preparing for apoptosis, were also positive for pre-$TGF\beta_1$.[13] These studies suggest that hepatocytes programmed for cell death synthesize (pre-) $TGF\beta_1$, which may act in an autocrine manner ("autocrine suicide"). Additionally, $TGF\beta_1$ may affect apoptosis in a paracrine fashion because nonparenchymal cells also synthesize $TGF\beta_1$.[33]

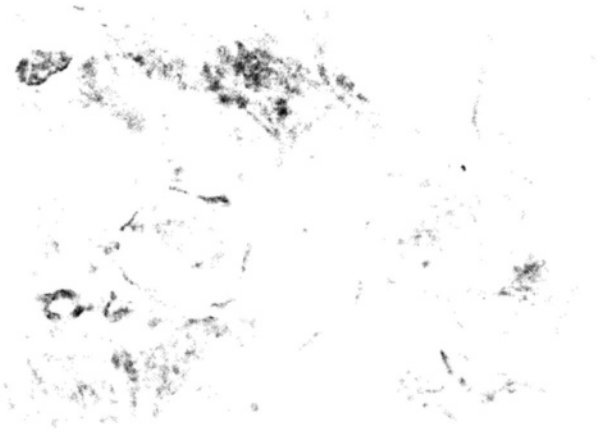

Figure 6. *Fas expression on the cell membrane of HCV-infected hepatocyte*

Increased expression of $TGF\beta_1$ at the protein level, detected by immunohistochemistry, was found to be correlated with the histological activity in CH.[78,97] The higher the grade of disease, the higher the $TGF\beta_1$ expression found.

Another signal system involved in induction of apoptosis is the cell surface receptor known as *Apo1, Fas* or *CD95* and its ligand. Fas belongs to the tumor necrosis factor receptor/nerve growth factor receptor family.[81] The ligand to the Fas/Apo1 receptor has been isolated and turned out to belong to the TNF/nerve growth factor family.[110] It is expressed in cytotoxic T-lymphocytes and induced death in cells expressing the Fas receptor (*Fig.6*), when FasL binds to Fas, the target cell undergoes apoptosis.[47] Activated T-cells are able to undergo autocrine suicide which may be important for suppression of immune response and peripheral tolerance by T-cell deletion.[27] The Fas ligand/receptor system represents one of two known mechanisms of cell killing by cytotoxic lymphocytes.[77] Various cells express Fas including hepatocytes, whereas FasL is expressed predominantly in activated T cells.

Cell-mediated immunity is important in acute and chronic hepatitis. Piecemeal necrosis occurs, where lymphocytes or macrophages and hepatocytes are in close contact. Cytotoxic T-lymphocytes and pit cells may induce apoptosis-like cell death in hepatocytes by either the perforin or the Fas ligand pathway.[1,16,18] In patients with CH-C, Fas antigen has been found to be expressed particularly at the advancing edges of piecemeal necrosis.[42] In another study, Apo1/Fas expression in the hepatocyte cell membrane was low in intact liver tissue, but elevated in cirrhosis and liver failure associated with HBV infection.[35] Fas ligand mRNA expression was not found in normal liver but was demonstrated in HBV and HCV-related liver

disease in areas with lymphocytic infiltration.[35,42] Data suggest that acute fulminant hepatitis in humans may be Fas-mediated and specific CTLs are involved in fulminant hepatitis.[1] Primary hepatocytes are sensitive to Fas-mediated apoptosis in vitro. Human hepatitis C virus (HCV) transformed hepatocytes overexpress Fas.[42]

In this model, virus antigens of hepatitis B virus or HCV expressed on hepatocytes would activate CTLs to express FasL, which then would bind to Fas on hepatocytes *(Fig.6)*, inducing them to undergo apoptosis. This process may normally occur to remove virus-infected cells but, if exaggerated, may lead to fulminant hepatitis. *(Fig.7)*

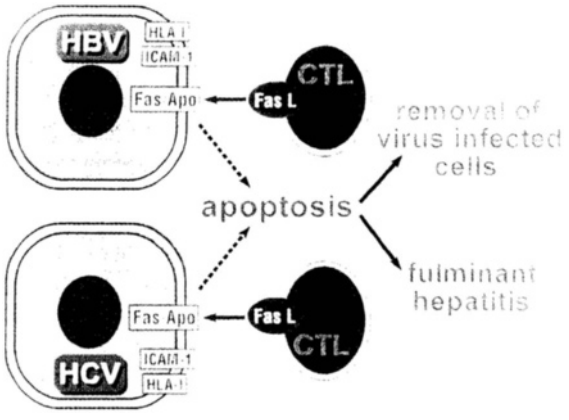

Figure 7. *Pathomechanism of hepatocyte death during viral infection.*

It has been shown that viral infection can inhibit transcription of cellular proteins and predispose cells to *TNF-induced death.*[36] The type of cell death induced by TNFα exhibits some morphological signs of *apoptosis*, but also shows pronounced *lytic* damage. Death of hepatocytes during viral infection can occur as a *direct* effect of the virus or as a result of the *host immune* response. Apoptosis may represent a host defense mechanism against viral infection by which the infected hepatocytes death prevents viral replication and spread.

Expression of viral antigens and nucleic acids in the liver

The expected complexity of the pathomechanism causing liver damage in hepadnavirus infection, the presumed leading pathogenetic role of viral antigens and the insufficient recognition of factors influencing the perpetuation and severity of hepatitis determine the need for careful delineation of the *expression of virus-encoded proteins.*[8,98] The determination of molecular species of viral polypeptides in HC-plasma membrane may also help to elucidate the pathway of intracellular processing of viral proteins and mechanisms of virion assembly.

The expression of viral antigens during hepatitis is the most extensively studied and known in HBV-infection.[8,36,43] Hepadnaviral antigens exposed on HCs serve as targets and as possible modulators of immunopathogenetic reactions causing liver damage.[71] Findings indicate (in woodchucks) that hepadnavirus core and envelope polypeptides are integral constituents of HC membranes in the course of hepatitis.

The early hypothesis that the immune response determines the morphological presentation and course of the non-cytopathic HBV infection has gained general acceptance.[8] There is a general agreement that the HBV is not cytotoxic under ordinary circumstances.[98] Data that HBV might be directly cytotoxic came from an in vitro study of HepG2 cells, in which accumulation of capsid proteins in nonproducer clone proved to be cytotoxic.[90] In another study, it has been demonstrated that deregulated expression of the large envelope protein of HBV can be directly cytotoxic in transgenic mice.[18] Overexpression of this viral protein leads to the formation of nonsecretory filamentous HBsAg that accumulates within the endoplasmic reticulum. At lower intracellular HBsAg concentrations however, the HCs appear to be functionally normal.[18]

It has been shown that HBsAg-positive transgenic HC is selectively sensitive to destruction by IFNγ in vivo.[36] Because this cytokine has been shown to be produced by intrahepatic lymphocytes during viral hepatitis in humans, these results raise the possibility that a similar pathway may contribute to the clearance of HBsAg-positive HCs and to the pathogenesis of liver cell injury in human HBV infection as well.[36]

Another exception to indirect cytoplasmic effect of HBV occurs with liver transplantation after which reinfection of the graft may be characterized by the rapid accumulation of large amounts of HBsAg and HBcAg without much infiltration by lymphoid cells causing cellular damage.[21]

Specific antibodies are used to visualize the viral proteins in *HBV-infection. HBcAg* is localized mainly in the hepatocyte nucleus and *(Fig.8)* to a lesser degree in the cytoplasm and/or in close association with the cell membrane. Intracellular, nonmembranous localization of the HBV core polypeptide in infected cells is not an integral membrane protein.[85] The cytoplasmic HBcAg is considered to represent active replication. Under the electron microscope, core particles look like non-coated 24-27 nm ring-shaped structures located in the karyoplasm and between the cisterns of the endoplasmic reticulum. Encapsulation of core particles takes place within the endoplasmic reticulum, forming the complete virus, the 42 nm size Dane particle. Excess accumulation of core particles can be observed in HE stained sections, as "sanded" nuclei.[8] Nuclear HBcAg probably represents accumulation of empty nucleocapsids whereas cytoplasmic HBcAg is considered active virus replication since viral DNA can be

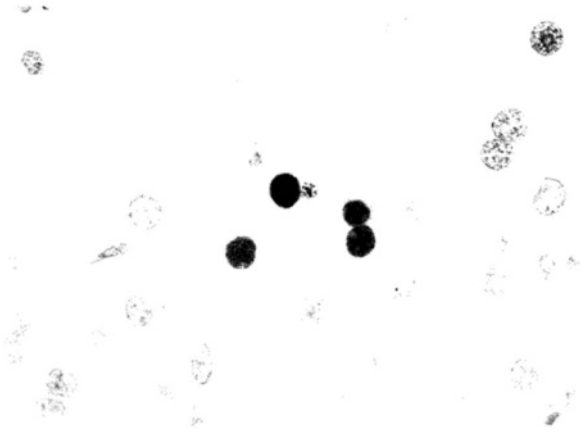

Figure 8. HBcAg in chronic carrier. Immunohistochemistry.

demonstrated by in situ hybridization in such liver cells.[8] HBsAg can be well demonstrated by immunohistochemistry in different patterns as membranous and intracytoplasmic HBsAg *(Fig.9)* can be stained by specific techniques (Shikata's orcein, aldehyde fuchsin, Victoria blue etc.) with varying density and extension in the cytoplasm. Excess cytoplasmic accumulation of HBsAg in the HC can be seen as a homogenous "ground-glass" appearance of the cytoplasm *(Fig.10)*.

Under electron microscopy, ground glass hepatocytes show a marked proliferation of smooth endoplasmic reticulum with typical filaments within the cisterns. Intracisternal HBsAg probably represents accumulation of the surface component which can not be secreted by the cell and represents a chronic elimination insufficiency for this antigen. This may or may not associated with core formation.[8]

Bianchi and Gudat[8] pointed out the importance of the *basic reaction types* of HBV-infection in the 70s and it has been incorporated in a recent modification. They distinguished the *elimination type* (acute self-limited VH), the *generalized HBcAg* type, the *focal HBc type* and the *HBcAg-free HBs type*.

Bianchi and Gudat[8] group the four basic reaction types in HBV-infection in a dynamic model suggesting a natural evolution of CH-B. The infection starts with a highly replicative phase and extensive cellular expression of viral proteins, mainly HBcAg. Within about six years, an increasing immune response results in eradication of some but not all HBcAg-bearing hepatocytes. This results in a reduction of HBAg expressing cells and increased inflammatory activity. The result is architectural disturbances, fibrosis, and even cirrhosis. Usually with partial elimination of replicating cells, the inflammatory response after some 4-6 years declines and shifts towards non-replicative phases and histologically minimal hepatitis or CPH.

The three chronic reaction patterns suggested by Bianchi and Gudat,[8] however, are not sharply delineated entities but characteristic types that merge into each other. Transitions may occur in any direction. Application and withdrawal of immunosuppressive and antiviral therapy could result in simultaneous transition both of histological findings and viral expression pattern. Under certain circumstances, however, the viral expression might be different. Uchida et al[115] published that certain cases of so-called type F acute and chronic hepatitis is caused by a HBV variant with mutations in the X open reading frame. This "silent" HBV mutant does not induce immunoserological markers. However, weak positive immunostaining for HBsAg and nuclear HBcAg was observed.

The mechanisms involved in HBV *persistence* are poorly understood, although they probably combine viral and host factors.[42,91] A role of defective particles in viral persistence has been suggested for several viruses. The mechanism involved in this modulation might implicate modification of viral proteins, viral virulence or alteration of the host immune response by modulating lymphocyte functions.[91] The expression of defective HBV (dHBV) DNA alone leads to a marked intracellular accumulation of the

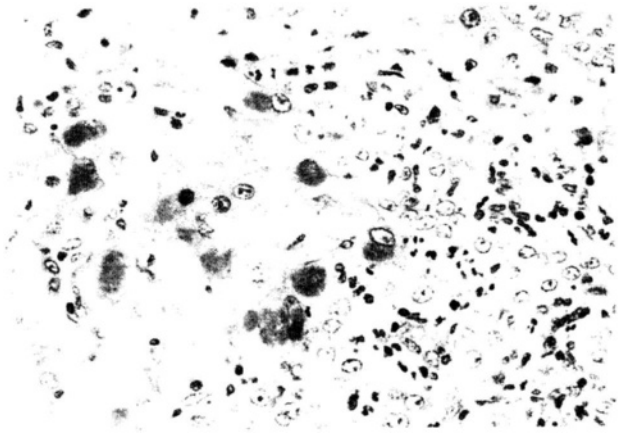

Figure 9. HBsAg in chronic hepatitis. Immunohistochemistry.

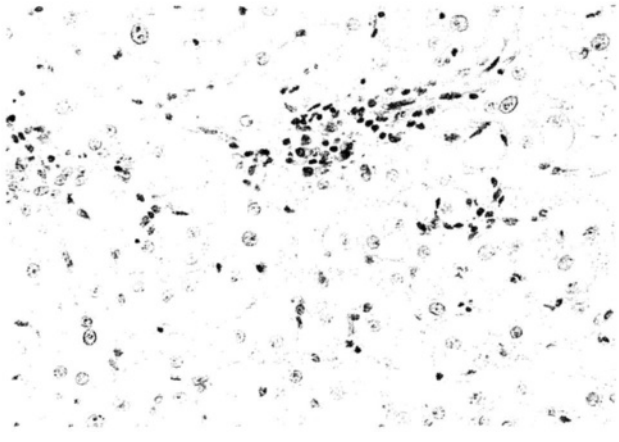

Figure 10. Ground glass hepatocytes (HE).

major core protein (HBcAg) and to an increased secretion of HBsAg. Spliced HBV RNA leads to the synthesis of proteins that might potentially modulate the viral replication or cytopathic effects. This data might be significant, because as it has been mentioned earlier, the accumulation of capsid proteins in the HepG2 cells may be directly cytotoxic;[90] and the increased secretion of HBcAg may favor immunotolerance to HBV particles.

Accumulation of the HBV RNA molecule coding for the X protein in dHBV DNA was found in transfected cells. This might be significant, because X protein has been shown to transactivate a variety of viral and cellular genes, including human lymphocyte antigens and adhesion molecule encoding genes.[45]

It is well known after the discovery of the Δ agent by Rizetto et al[89] that the *hepatitis D virus* (HDV) co-exists with and depends on replicating HBV infection.[23] Immunohistochemically, HDAg is found mainly in hepatocyte nuclei, with different patterns.[53,70,79] Excess HDAg may causes "sanded" nuclei in HE stains similar to HBV infection. The pioneering work of the Turin group[89] has shown mostly separate expression of HDAg versus HBsAg or HBcAg (if present at all) but co-expression in the same cell does occur with HBsAg and, rarely, with HBcAg *(Fig.11).*

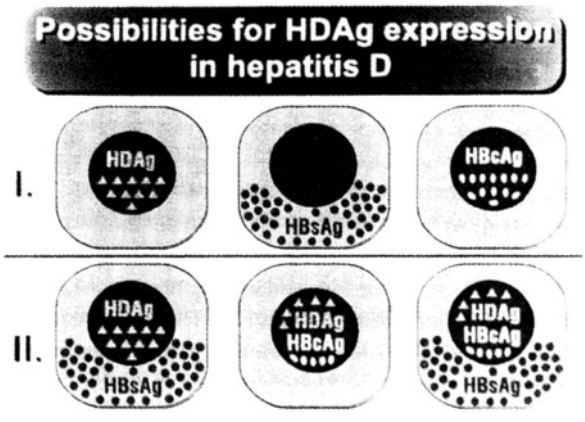

Figure 11. *Possibilities for HDAg expression in hepatitis D.*

The pathogenesis of hepatitis D is still unclear despite considerable progress in the understanding of the molecular biology of the virus. In certain circumstances, HDV infection causes a mild disease, while, in others, it induces aggravated and accelerated disease leading to cirrhosis more frequently and in a shorter time, especially in drug addicts or in hyperendemic regions.[21] In other areas a carrier state with low grade HDV replication and lack of tissue damage has been found.[89]

HDV is cytotoxic, in contrast to HBV, producing direct cellular damage in addition to immunologically mediated cellular cytotoxicity.[22]

Figure 12. *HCV-antigen (NS4) in chronic hepatitis detected by immunogold method.*

HDV rather selectively suppresses HBcAg and HBeAg and not HBsAg or pre-S, although it supposedly depends on minimal HBV multiplication and release.[109] This may lead to HBV expression patterns in liver and blood which deviate from those of uncomplicated chronic hepatitis B.[8]

The identification of the genetic organization of *hepatitis C virus*[20] provided the basis for further studies in this field.[19,30,32,39,48,49,52,58,61,68,75,81,87, 95,106,107] Several groups have demonstrated hepatitis C virus (HCV) antigens and HCV genome in the liver, but the techniques and reagents are not currently suitable for routine use.[10,29,31,38,55,59,60,76,80,92,93,94,105]

Immunohistochemical localization of the HCV nonstructural Ag (NS4) was detected in frozen and formalin-fixed human biopsy samples within the cytoplasm of HC *(Fig.12),* but not in the mononuclear cell infiltrates, bile duct epithelium or endothelial cells.[9] A high proportion of HC were positive (60-90%), but the staining intensity was variable.[9]

In another study[28] positive staining for HCV-Ag appeared to correlate well with the presence but not the degree of HCV replication as measured by HCV-RNA.

Haruna et al[10] used liver and hepatocellular carcinoma tissue obtained from partial hepatectomy specimens or from livers removed at transplantation of chronic HCV infected patients. Monoclonal antibodies directed against the core, envelope, NS3 and NS5 antigens of HCV were used on frozen samples and granular or homogenous cytoplasmic immunostaining was observed. Hiramatsu et al[41] detected strong positive cytoplasmic immunostaining for core, envelope and NS3 antigens of HCV at different levels. Komminoth et al[52] used a commercially available antibody against NS proteins (C-100) (TORDJI-22, Clonatec, Paris) and found positivity both in HCV infected and non-infected liver tissue. Krawczynski et al[57] detected HCV non-structural Ags (NS3/4, NS3, NS4, NS4/5) and structural antigens (core, envelope) in chimpanzee and human livers infected with HCV by immunofluorescence.

HCV Ag had a fine granular or powder-like fluorescence pattern with larger, superimposed granules. Hepatocyte nuclei were always negative for HCV Ag. HCV Ag was never found in non-parenchymal cells in bile duct epithelium. Sansonno and Dammacco[94] observed NS3/NS4 products in the cytoplasm of HCs in HCV infection. No membranous accumulation was seen.

Immunostaining with anti-E_1 and -E_2 antibodies was observed in cells transfected with HCV. The pattern of fluorescence is consistent with a location within the endoplasmic reticulum. C-100 protein (NS4) was demonstrated in the endoplasmic reticulum.[103]

The identification and immunostaining of proteins encoded by the HCV genome suggest that most of them reside in the endoplasmic reticulum and only NS3 and NS5a may be soluble.[103] The observed cytoplasmic location of core and to some extent NS5, proteins might serve to identify the sites of viral assembly.

HCV-NS5 Ag was detected in fine granular distribution in the cytoplasm of HC, but not in the nucleus or cell membrane. By IEM, the Ag was observed along the endoplasmic reticulum but not in other organelles.[114] The NS5 was detected in 52% of CAH-C, in 33% of CPH and 86% of cirrhosis cases. The different expression patterns which are so well documented in HBV and HDV infection are not so well described in HCV infection. It has been shown that HCV core protein followed by NS4 are the most potent T cell immunogens for both CH-C patients and asymptomatic anti-HCV-positive subjects. Others found the NS4 to be the most immunogenic HCV antigen in CH-C.[11]

It has been suggested that liver injury in CH-C is correlated with the persistence of detectable HCV-RNA in serum.[95] However, the presence of HCV RNA in asymptomatic individuals indicates that some immunological mechanisms may be also be pathogenetically relevant. The lowest levels of HCV viraemia was found associated with minimal liver disease.[37]

Host immune response to hepatotropic viruses

It is assumed that liver injury in VH is mainly a consequence of host cytotoxic immune responses directed to the virus-encoded products rather than the result of a direct cytopathic effect.

The predominance of *activated T cells* in inflammatory periportal infiltrations *(Fig.13)* and the strong expression of *HLA class I and class II* molecules on HCs and T cells in CH suggest that the hepatic injury is mediated by viral Ag-specific immune reactions.[8] $CD8^+$ cytotoxic T cells simultaneously recognize both HBV-related antigens and HLA class I Ags expressed on the HC membrane and then attack and destroy the infected HCs. Besides Ag-specific HLA-class restricted cellular cytotoxicity, *natural killer cells, lymphokine-activated killer cell* activity may play an additional role in the pathogenesis of CH.[112] *Cytokines* released by activated T cells may be crucial for the development of CH.[65] So far, an increase of IFNγ secretion has been found after stimulation of peripheral blood mononuclear cells with recombinant HBV nucleocapsid antigen in CH-B. Others showed that T cell lines in CH-B and CH-C secrete large amounts of TNF-α, IFNγ and IL-2 after mitogen stimulation.[65] These cytokines have a wide effectory system, including direct cytotoxicity, maturation of precursor cells, induction of expression of HLA-molecules, activation of bystander cells and increased susceptibility for NK cells.[65] More recently it has been reported that local production of IFNγ maintains, a liver-specific inflammatory disease in transgenic mice models.[113]

It has been suggested by Desmet[26] that the central core of the portal inflammation in CH-B represents a B-cell zone and the peripheral invading front of the piecemeal necrosis corresponds to a T-cell zone, as in lymph nodes. The same has been found by Bianchi and Gudat.[8] Indeed, *lymphoid follicle* formation is seen quite often at the center of the portal and periportal infiltrate with piecemeal necrosis, especially in CH-C infection.[74] By in situ immunophenotyping, it has been shown that lymphoid follicles with activated B cells in germinal centers are surrounded by a follicular dendritic cell network. A T-cell zone comprising CD4-positive helper T cells, CD8-positive suppressor/cytotoxic T cells was seen at the periphery of the nodules *(Fig.13)*.[74]

It has been found that the intrahepatic lymphoid follicles are mainly primary, without germinal centers and contain a very low number of follicular dendritic cells.[34] This raises the possibility that intrahepatic lymphoid aggregates represent a site of replication of the virus, based on the observation that HCV virus can infect mononuclear cells in the peripheral blood. Ando et al[1] points out the fact that the CTLs have direct and immediate access to the target HCs, which they have the poten-

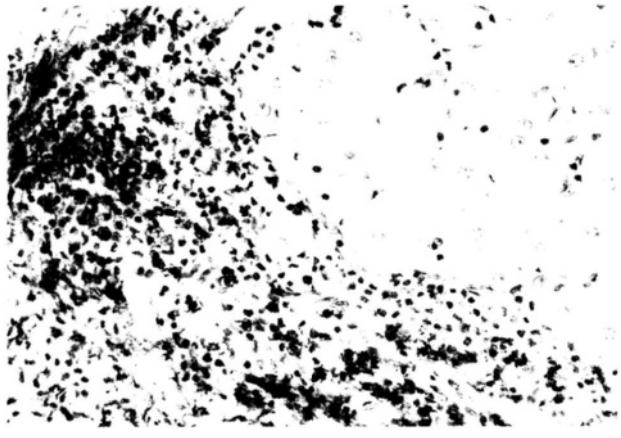

Figure 13. CD8 lymphocytes in the portal and periportal infiltration. Immunohistochemistry.

tial to kill, because of the unique microanatomy of the sinusoids. These contain a discontinuous endothelium and lack a basement membrane. T-lymphocyte response to viruses may have two opposing affects. On one hand, they may be critical for protection either directly through $CD8^+$ T-killer cells or indirectly through $CD4^+$T cells, which help B lymphocytes to produce neutralizing antibodies. On the other hand, T cells may be harmful, mainly through $CD8^+$ T cells, which damage infected tissues in an attempt to clear the virus.[11]

The correlation between HLA molecules and HBV-infection has been studied extensively. Using a model in which HBsAg-specific CTL caused an acute necroinflammatory liver disease in HBsAg transgenic mice, it has been demonstrated that class I-restricted disease pathogenesis is an orderly, *multistep process*, that involves direct, as well as indirect consequences of CTL activation. The most destructive pathogenic function of the CTL, however, is to secrete IFNγ when they encounter antigen in vivo, thereby activating the intrahepatic macrophages and inducing a delayed-type hypersensitivity response that destroys the liver and kills the mouse.[1] This model or hypothesis solves the problem that apoptosis itself is usually not a proinflammatory event. The second and third steps suggested are responsible for the inflammatory reactions and can be considered as independent indirect functions of the CTL that are mediated by IFNγ and other currently unidentified cytokines. This model corresponds well with the hypothesis of the relationship between cell-mediated immune attack, hepatocyte death and fibrosis in CAH, suggested by Kerr et al in 1979.[50]

HBV infection increases the display of HLA class I heavy-chain and β2-microglobulin antigens on HCs and it correlates well with histological activity; β2 microglobulin expression was more intense in areas of piecemeal and lobular necrosis in close association with inflammatory T-cell infiltrates. In another study on a hepatoblastoma cell lines, it was found that HBV replication inhibits expression of HLA class I on infected hepatocytes. This effect of HBV replication on the host cell may be a means by which HBV evades immune surveillance to maintain chronic infection.

Less data are available on CH-C and HLA expression. It has been shown that hepatocellular HLA-A, B, C expression before IFN treatment is significantly higher in HCV patients who will not respond to IFN treatment than in responders. It had been suggested that the higher hepatocellular expression of class I MHC molecules in nonresponder cases may reflect a different viral effect on HCs, which is induced by different HCV genotypes or levels of viraemia.[5] HCV-specific, HLA class I-restricted CTL response was demonstrated in liver-infiltrating lymphocytes from persons with CH-C.[56]

It has been suggested that *viral sequence variations*[14,20,30,49,52,68,75,82,115] lead to escape from cellular immune recognition, and contributes to the development of persistent HCV-infection.

Coexpression of HLA and adhesion molecules such as *intracellular adhesion-1 molecules (ICAM-1)*, is necessary to obtain HC and T cell contacts. Normally ICAM-1 molecules are detected in sinusoidal cells and vascular endothelium and HCs, however, bile duct cells are negative.[5] In diseased liver, an increased intensity of immunostaining of HLA-A, B, C and ICAM-1 molecules was observed at the HC membranes.[5] Double immunofluorescence has shown that HCs containing HCV antigens were also positive for both ICAM-1 and HLA-A, B, C.[5]

Viral *persistence* is associated with the development of mechanisms for avoiding viral recognition by host cytotoxic T cells. Down-regulation of HLA-A, B, C and ICAM-1 molecules is a well-documented *escape mechanism* of hepatic and nonhepatic viruses.[5]

In summary, the pathogenetic mechanisms responsible for the *final outcome* of hepatotropic viral infection is still under study and discussion. Several factors, such as viral factors (genotype, mutation), virus host cell interaction, expression of viral proteins, virus – host immune response to antigen recognition, several cytokines etc. play an important role in the pathogenesis and finally lead to the well known histological alterations of viral hepatitis.

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