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Liver Lesions in Hepatitis B Viral Infection

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A review is made of the various histological lesions observed in hepatitis B virus-related liver diseases, including different forms of acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The elementary lesions discussed include acidophil necrosis (apoptosis), confluent lytic necrosis in its different patterns, piecemeal necrosis, focal necrosis, and dysplastic hepatocytes. Their pathogenesis is explained in the framework of recent developments in the immunopathology of hepatitis B viral infections.

INTRODUCTION

Infection with the hepatitis B virus (HBV) results in a broad variety of clinical diseases. Evidence supporting this clinical experience came from transmission studies in volunteers nearly half a century ago, in which identical inocula of HBV were injected into healthy recipients and resulted in different clinical responses [1,2].

In 1972, it was first suggested [3] that HBV is not directly cytopathic to the hepatocyte, and that hepatic inflammation and liver cell damage are mediated by various immunological mechanisms. The thesis that host immunity is most important in the causation of liver damage has gained considerable support [4,5], although the factors which determine a particular syndrome in the individual patient remain largely unknown, and direct cytotoxicity of the virus on liver parenchymal cells is not excluded [6,7].

The various hepatic diseases which may result from HBV infection are shown in Fig 1. This paper reviews the main histopathological liver lesions and their presumed pathogenic mechanisms.

ACUTE VIRAL HEPATITIS B

Acute viral hepatitis B may take the form of a mild disease, either subclinical, anicteric infection or classical icteric hepatitis with spontaneous recovery, or it may present more rarely as a severely necrotizing, life-threatening disease.

All forms of acute viral hepatitis B apparently result from an adequate response of

Abbreviations: HBcAg: hepatitis B core antigen HBsAg: hepatitis B surface antigen HBV: hepatitis B virus Lex: liver extract LIP: liver immunoregulatory protein *or* liver-derived inhibitory protein LMA: liver membrane antigen LSP: liver specific protein MHC: major histocompatibility complex NK: natural killer RIF: rosette inhibitory factor

This lecture was presented on April 16, 1987, to inaugurate the Annual Gerald Klatskin Memorial Lectureship in Hepatology. The Klatskin Lectureship has been established by the Department of Internal Medicine through gifts from former fellows, family, and friends, in memory of Dr. Gerald Klatskin (1910–1986), founder of the Liver Study Unit at the Yale University School of Medicine and a member of the Department of Internal Medicine for more than 50 years.

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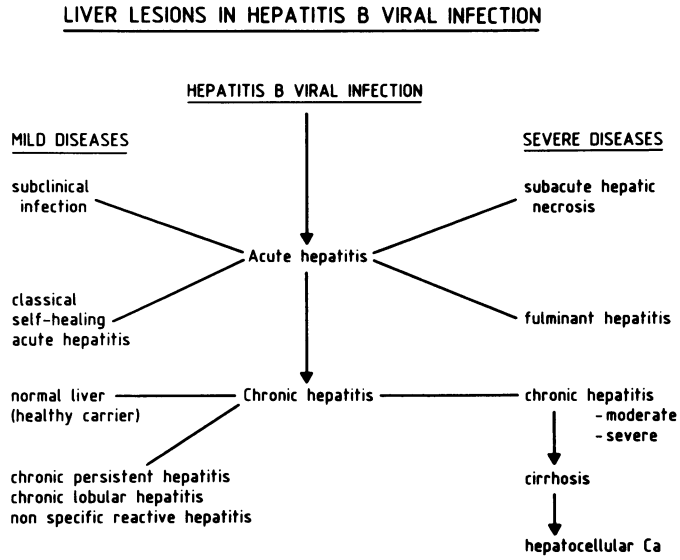

FIG. 1 Liver lesions in hepatitis B viral infection (modified after Thomas et al. [5]).

the host's immune defense, resulting in complete elimination of the virus; acute viral hepatitis B thus represents an "elimination type" of hepatitis [8]. Eradication of the virus implies, however, elimination of virus-infected hepatocytes; this fact is reflected in the complex array of histological lesions observed, including parenchymal damage, hepatocellular necrosis, liver cell regeneration, and inflammatory infiltration.

CLASSICAL ACUTE HEPATITIS WITH RECOVERY

The histological lesion in uncomplicated acute hepatitis B is a complex one. The sinusoids are crowded with increased numbers of mononuclear cells, mainly macrophages and lymphocytes, with highest density in acinar zone 3. This increased sinusoidal cellularity reflects the intrahepatic immunological reaction against the virus. The sinusoidal lining cells and macrophages have been shown to express strongly HLA-DR or HLA class II antigens [9]. These cells are presumed to function as antigen-presenting cells to T-helper lymphocytes [10].

In order to react to the foreign viral antigen, T-helper lymphocytes need to "see" this antigen in conjunction with self antigens (HLA class II antigens) expressed on the surface of "accessory" or antigen-presenting cells (often indicated by the vague term "macrophages"). The latter secrete the lymphokine interleukin 1, which activates the helper T lymphocytes (Fig. 2). Activated T-helper lymphocytes in turn activate cytotoxic T cells and B lymphocytes through secretion of the lymphokine interleukin 2 (Fig. 2).

Infected hepatocytes supporting viral replication express part of the viral antigens at their plasma membranes: both hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) have been demonstrated on the surface of mechanically isolated hepatocytes in acute viral hepatitis B [11]. In addition to expressing viral antigens, the hepatocyte is also triggered to display at its surface HLA A,B,C (or HLA class I) antigens, which are not expressed on liver parenchymal cells in the normal state [9,12,13].

In this way, the stage is set for a cytotoxic T-cell attack to viral antigens expressed on

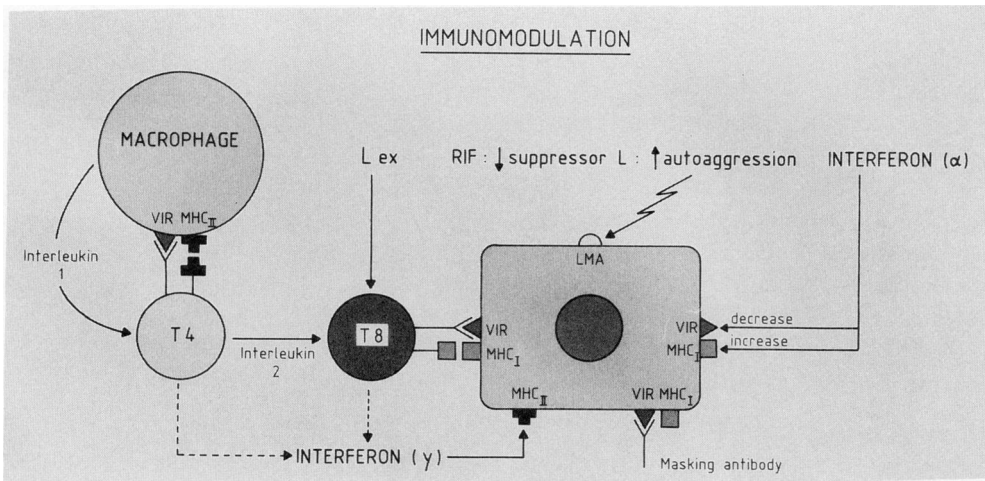

FIG. 2. Abbreviations: **LMA**: liver membrane antigen **L ex**: liver extract **MHC_I**: major histocompatibility complex antigens, class I **MHC_{II}**: major histocompatibility complex antigens, class II **VIR**: viral antigen **RIF**: rosette inhibitory factor **T4**: helper-inducer lymphocyte **T8**: suppressor-cytotoxic lymphocyte For explanation, see text.

the liver cell's surface, since cytotoxic T cells can now "see" the foreign viral antigen to which they are sensitized in conjunction with the HLA class I antigens. The latter is a necessary constraint of major histocompatibility complex (MHC) compatibility in order to assure their effective reaction [14,15].

On the basis of published histological and immunohistochemical observations, it appears that immunologic elimination of HBV-infected hepatocytes might be mediated by cytotoxic T lymphocytes. Such a mechanism was demonstrated in an elegant animal model of mouse hepatitis [16], and evidence in support of similar mechanisms in human acute viral hepatitis B was recently produced by functional immunologic studies [11].

Immunohistochemical analysis of lymphocyte subsets in acute viral hepatitis B revealed, however, that the infiltrating lymphocytes are not exclusively composed of OKT8+ cytotoxic T lymphocytes; the predominant lymphocyte subset corresponds to natural killer (NK) lymphocytes, which show no genetic restriction and do not require HLA class I antigen display on the target cells [17]. Also, recent functional immunological studies suggest involvement of NK cells [11,18]. These observations fit with what is known in other viral infections, that NK cells are the first line of defense against viral infection.

The result of the lymphocyte attack—either by cytotoxic T cells or natural killer cells or both—is a killing of the target cell.

The mode of cell death in immune-mediated target cell lysis is not classical cell necrosis, but apoptosis [19]. Apoptosis is a special pre-programmed and active mode of cell death. A precise sequence of intracellular changes occurs, including condensation and margination of the nuclear chromatin and formation of numerous blebs at the cell surface, but the intracellular organelles remain well preserved in contrast to what happens in classical cell necrosis. Finally, the cell breaks down into several fragments surrounded by a membrane.

Under the light microscope, apoptosis can be recognized as smaller and larger eosinophilic cell fragments, occasionally containing a pycnotic nucleus (Fig. 3). These

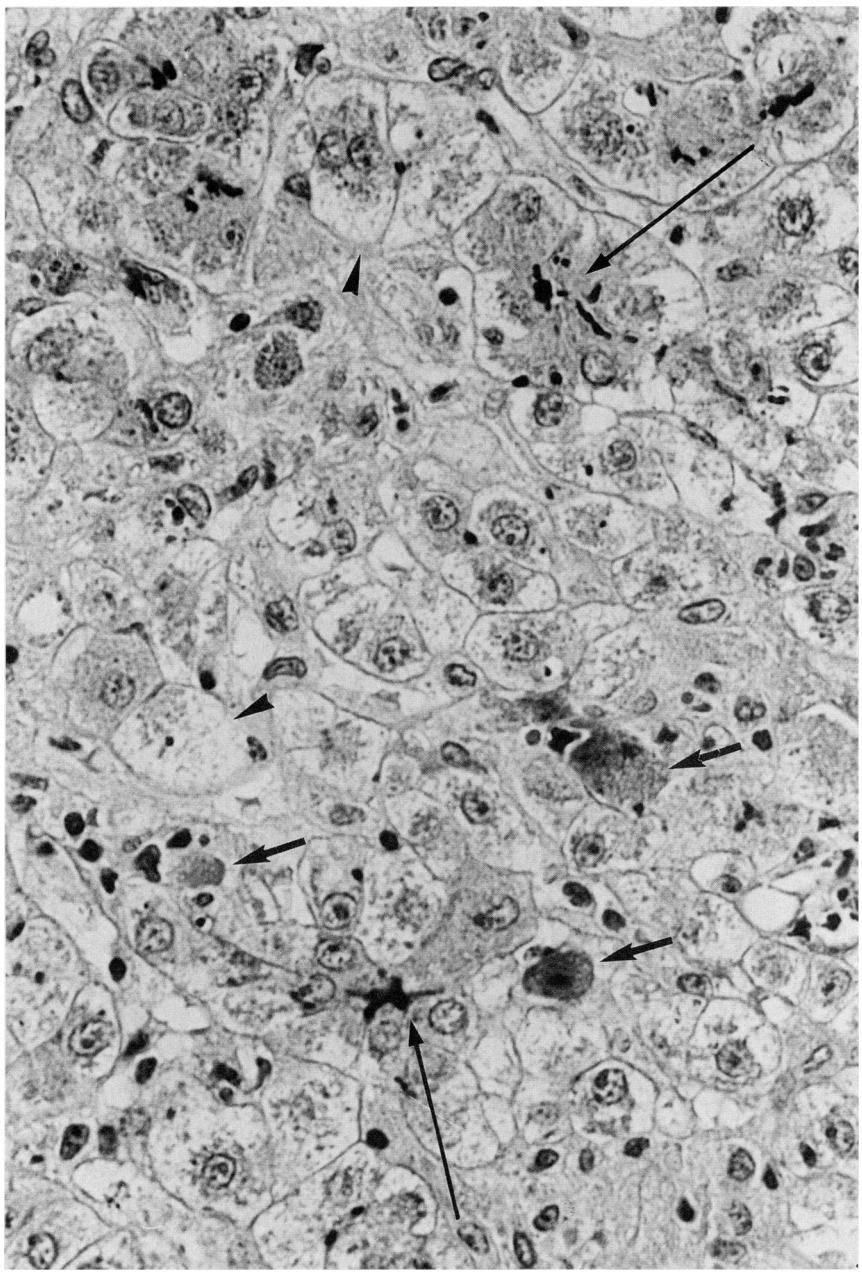

FIG. 3. Centrolobular (zone 3) parenchyma in acute self-limited viral hepatitis B. Several acidophil (apototic) bodies are present *(arrows)*, some of them in close contact with lymphocytes. Some hepatocytes appear swollen (liver cell ballooning) *(arrowheads)*; intercellular bile thrombi *(long arrows)* indicate cholestasis. Hematoxylin and eosin, ×640

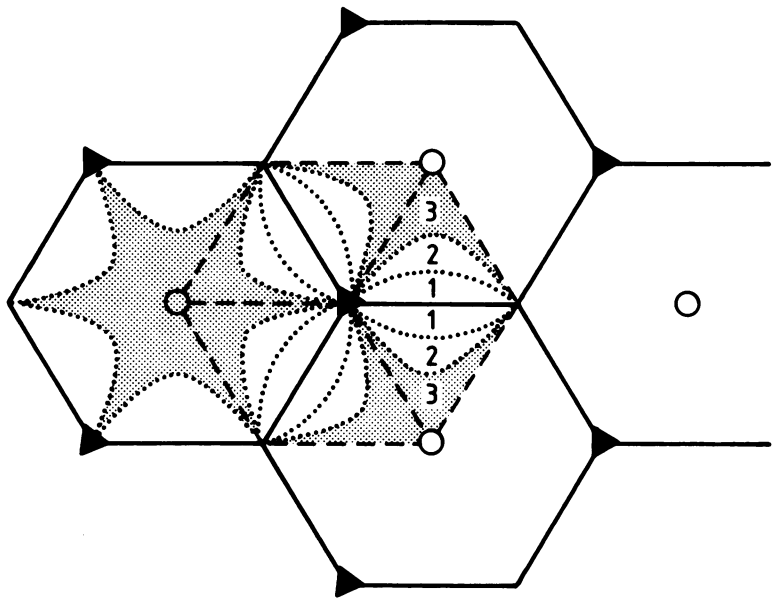

FIG. 4. Schematic drawing of the acinar and lobular concepts of liver architecture. Black triangles represent portal tracts, open circles central veins. The central hexagon, delineated by interrupted lines and centered by a portal tract, represents a complex acinus, composed of three simple acini which are centered by a terminal portal venule *(continuous line)*. Each simple acinus can be subdivided in acinar zones 1, 2, and 3, the latter representing the microcirculatory periphery. Necrosis in the periphery of the complex acinus leads to central-central bridging necrosis. Necrosis in the periphery of the simple acinus (zone 3) leads to portal-central bridging necrosis *(shaded area)*. This pattern of necrosis creates a star-shaped area of necrosis from the point of view of the classical hexagonal liver lobule *(left hexagon, centered by central vein)*.

apoptotic bodies become phagocytosed very soon by neighboring parenchymal cells and by nearby macrophages [20]. Close inspection of liver biopsies taken in patients with acute viral hepatitis B reveals numerous apoptotic bodies in close relationship with lymphocytes. Obviously, it is more difficult to identify the smaller apoptotic bodies, whereas the larger apoptotic fragments are easily recognized. The latter were known for many years as "acidophil necrosis," "acidophil bodies," or "Councilman-like bodies" [21] (Fig. 3).

Research on apoptosis has revealed that what was usually considered as single-cell necrosis does not at all correspond to classical cell necrosis but instead represents a lymphocyte-induced triggering of a pre-set program for active self-destruction of the liver cell [22].

Activated lymphocytes release several lymphokines; one of these soluble factors is a cholestasis-inducing lymphokine [23]. This finding may at least in part explain the occurrence of microscopic features of cholestasis in most liver biopsies from patients with acute hepatitis B (Fig. 3).

Not all damaged hepatocytes appear as small, shrunken apoptotic bodies in acute viral hepatitis B. One of the most striking features of the histopathological picture is liver cell pleomorphism, most pronounced in acinar zone 3 [21]. Several hepatocytes

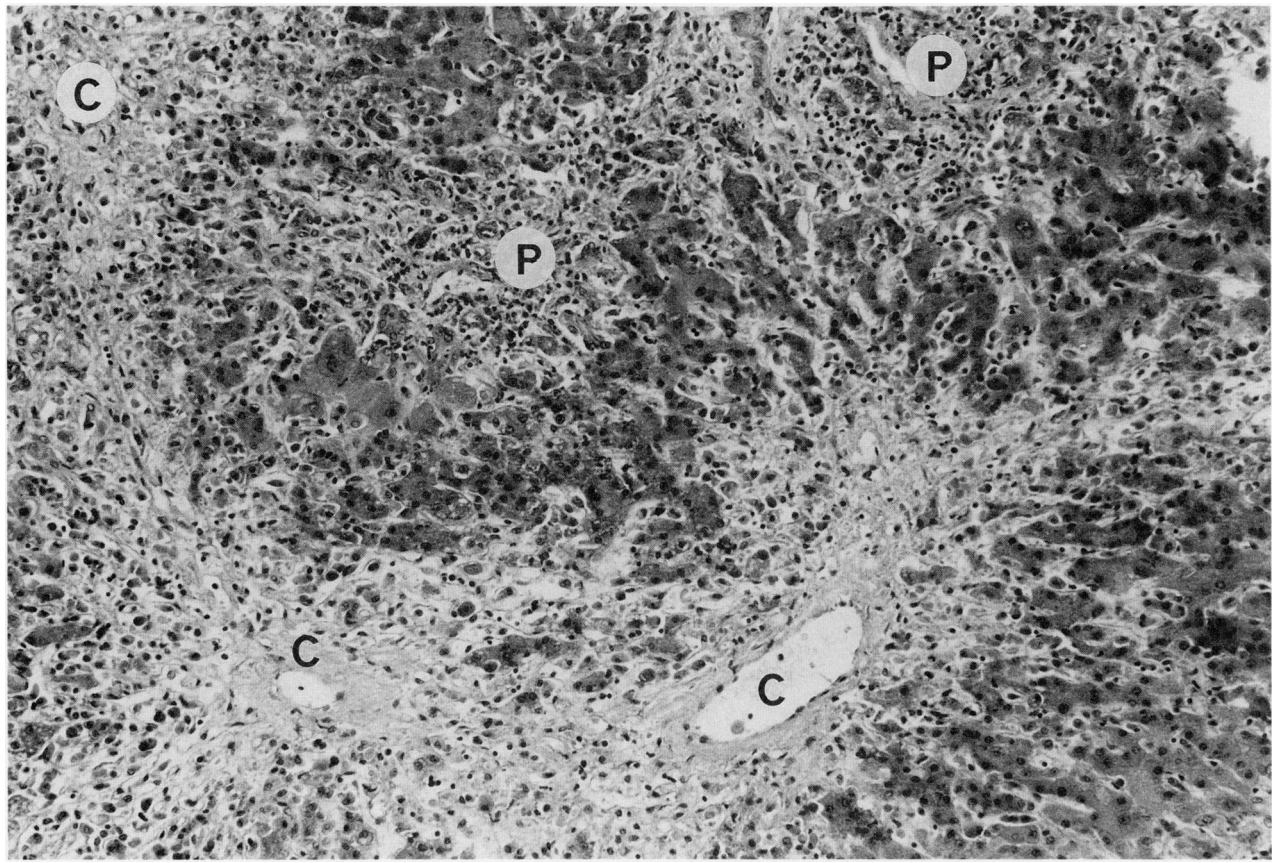

FIG. 5. Central-central bridging confluent necrosis. Autopsy specimen from patient with fulminant acute hepatitis B. An area of confluent lytic necrosis links several adjacent central veins (**C**); the portal tracts (**P**) show lymphoid infiltration and are surrounded by surviving parenchyma (complex acinus). Hematoxylin and eoxin, ×160

appear enlarged and pale, corresponding to "hydropic swelling" or "ballooning of hepatocytes" (Figs. 3,7). Hydropic swelling is believed to be a precursor stage of lytic necrosis of hepatocytes. The precise mechanism of this type of hepatocellular damage and death is not well known, but there is reason to assume that the primary damage is at the cell membrane [24]. It is this variety of cell damage with leaky cell membranes, which probably is most responsible for the release of intracellular enzymes and which contributes to the rise of serum transaminases.

Lytic necrosis of hepatocytes is a rapid event; the cell disappears in a very short time. Hence it follows that lytic necrosis is recognized histologically as an absence of cells. This condition is often described as "drop-out of liver cells," resulting in denudation of the reticulin framework. Lytic necrosis usually affects groups of hepatocytes, leading to the disappearance of confluent areas of parenchyma; this fact explains the term "confluent necrosis" [25].

SEVERE ACUTE VIRAL HEPATITIS B (SUBACUTE HEPATIC NECROSIS; FULMINANT HEPATITIS)

Confluent necrosis may affect smaller or larger parenchymal territories. When confluent necrosis destroys larger portions of parenchyma, the acute hepatitis is usually of a clinically more severe type, designated as subacute hepatic necrosis and fulminant hepatitis (Fig. 1). One of the remarkable features of confluent lytic necrosis is that it occurs in well-defined areas of the liver parenchyma: it shows a predilection for the microcirculatory periphery [26].

The microcirculatory periphery of the liver parenchyma is better explained by the acinar concept of liver architecture, according to Rappaport, than by the classical lobular concept according to Kiernan [27] (Fig. 4).

Confluent lytic necrosis in the microcirculatory periphery of the complex acinus leads to necrotic bridges between adjacent central veins (central-central bridging necrosis) (Figs. 4,5); whereas confluent necrosis in the microcirculatory periphery of the simple acinus (or acinar zone 3) creates necrotic bridging between portal tracts and central veins (portal-central bridging necrosis) (Figs. 4,6,7). The latter corresponds, at the lobular level, to a star-shaped area of necrosis linking portal tracts and central veins [27] (Figs. 4,6).

These patterns of necrosis correspond to what is often termed "bridging hepatic necrosis" [28]. When still larger masses of parenchymal cells succumb to confluent lytic necrosis, the drop-out of liver cells extends to zone 2 (Fig. 8), and even up to zone 1 of the simple acini, resulting in total destruction of all parenchyma of the liver units: panlobular and multilobular necrosis (Fig. 9). Very often, multilobular necrosis displays an irregular distribution throughout the liver, apparently corresponding to total destruction of some acinar agglomerates, whereas adjacent larger parenchymal units (or acinar agglomerates) are spared.

This predilection of confluent lytic necrosis to occur first in peripheral areas of the microcirculation, the apparent relationship of multilobular necrosis to hepatic angioarchitecture, and the relative scarcity of lymphocytes in the denuded areas are features which suggest that in this mode of necrosis other mechanisms are involved than those in focal apoptotic cell death.

The most plausible mechanism is a humoral one, possibly circulating antibodies or immune complexes, associated with activation of complement. Complement components (the complement C5b-9m complex) has been shown to cause a lytic type of

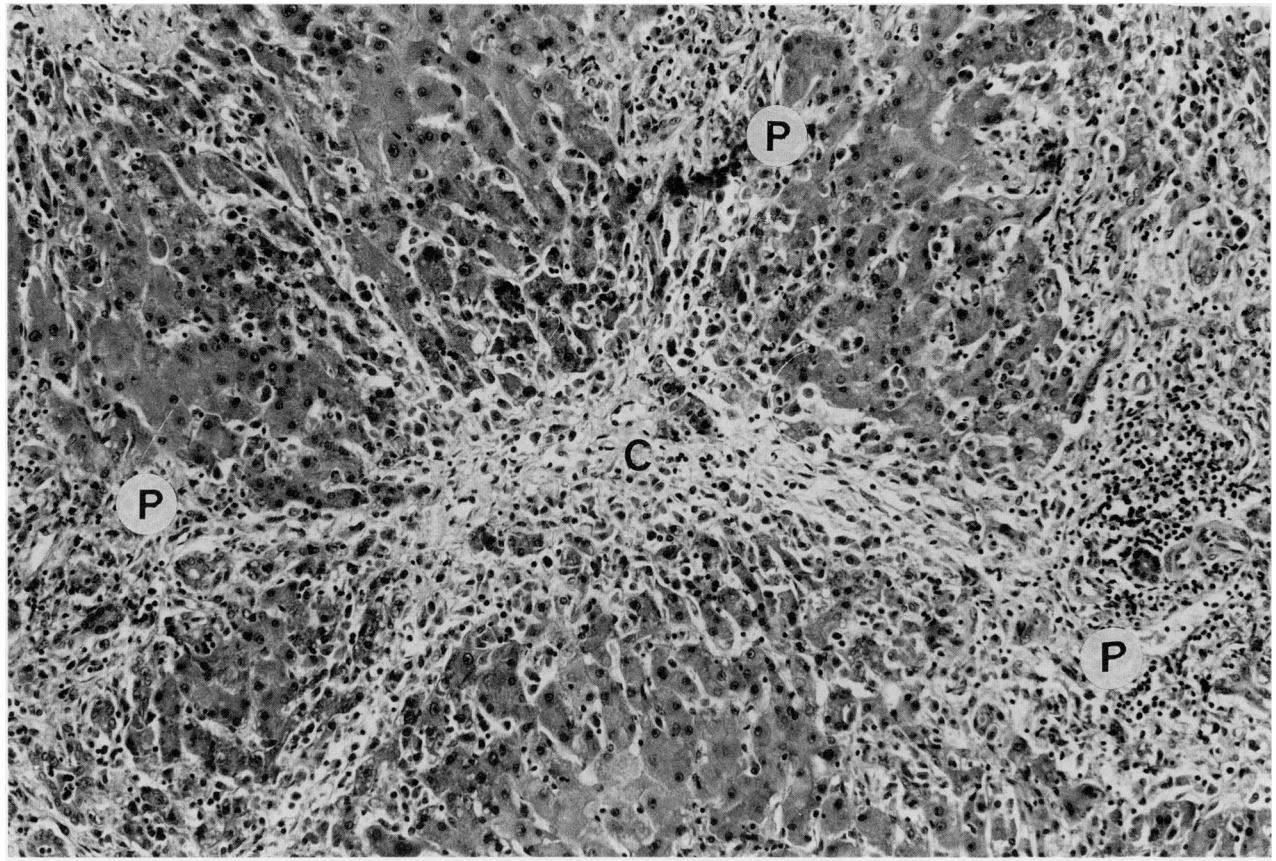

FIG. 6. Portal-central bridging confluent necrosis. Autopsy specimen from patient with fulminant acute hepatitis B (same case as Fig. 5). An area of confluent lytic necrosis links the central vein (C) with surrounding portal tracts (P). Portal-central bridging necrosis creates a star-shaped area of necrosis in the classical hexagonal liver lobule (compare with Fig. 4). Hematoxylin and eosin, ×160

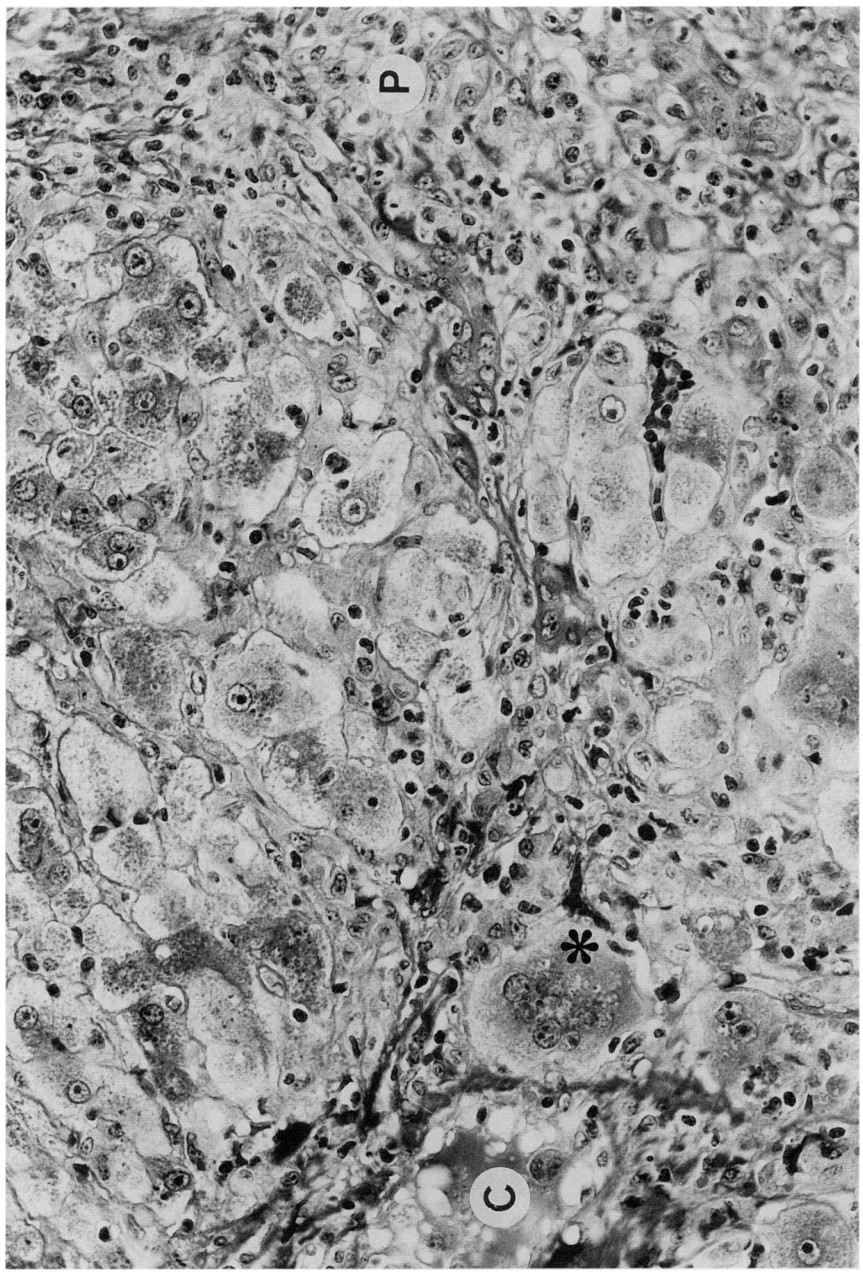

FIG. 7. Portal-central bridging necrosis. Liver biopsy from patient with acute hepatitis B. A streak of necrosis extends from the central vein (C) to the portal tract (P), interrupting parenchymal continuity. Note lymphocytic infiltration and ballooning of surviving hepatocytes. A multinucleated hepatocyte (*) near the central vein represents an attempt at regeneration. Masson's trichrome stain, ×400

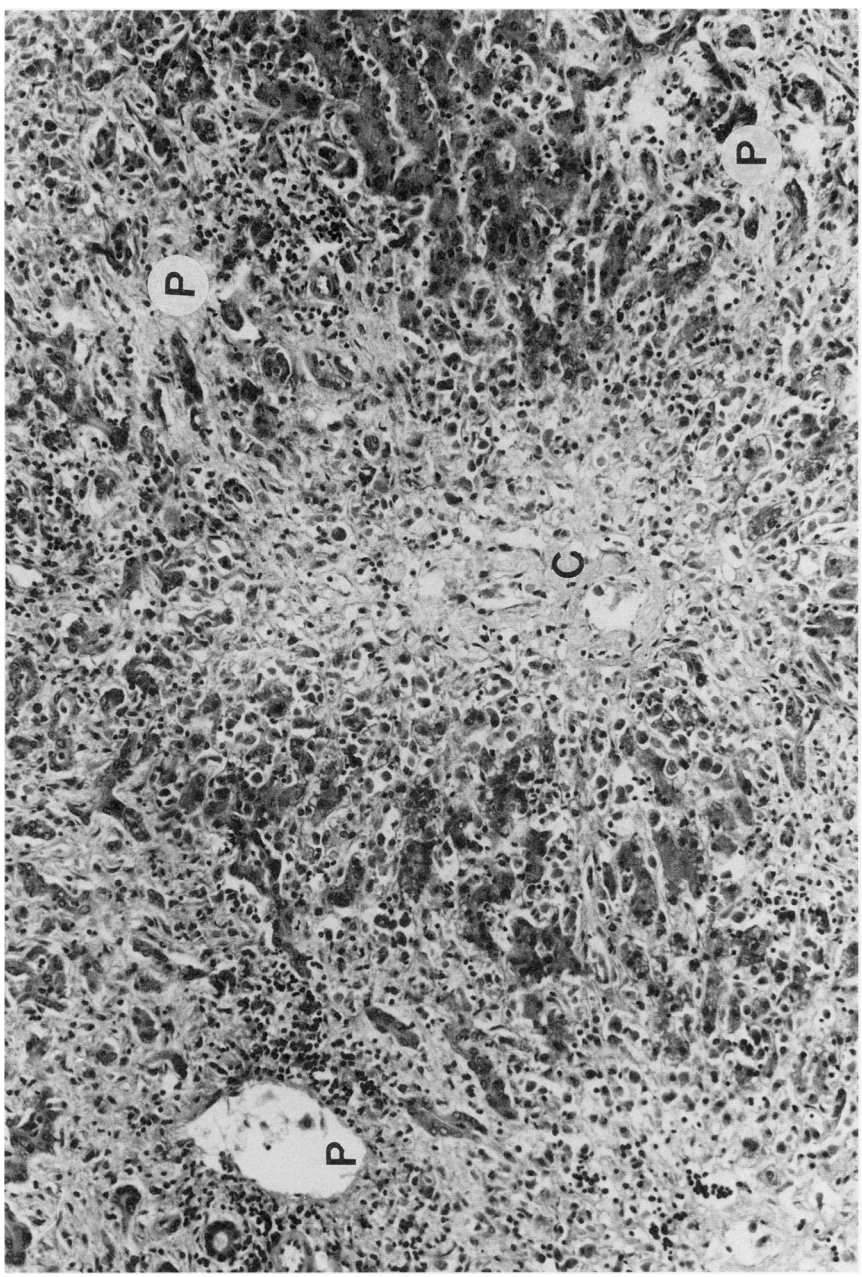

FIG. 8. Submassive confluent lytic necrosis. Autopsy specimen from patient with fulminant hepatitis B (same case as Fig. 5). Most of the liver lobule (acinar zones 2 and 3) is necrotic; the surviving parenchyma (zone 1) lies near the portal tracts (P). Early condensation of mesenchyme occurs around the central vein (C). Hematoxylin and eosin, ×160

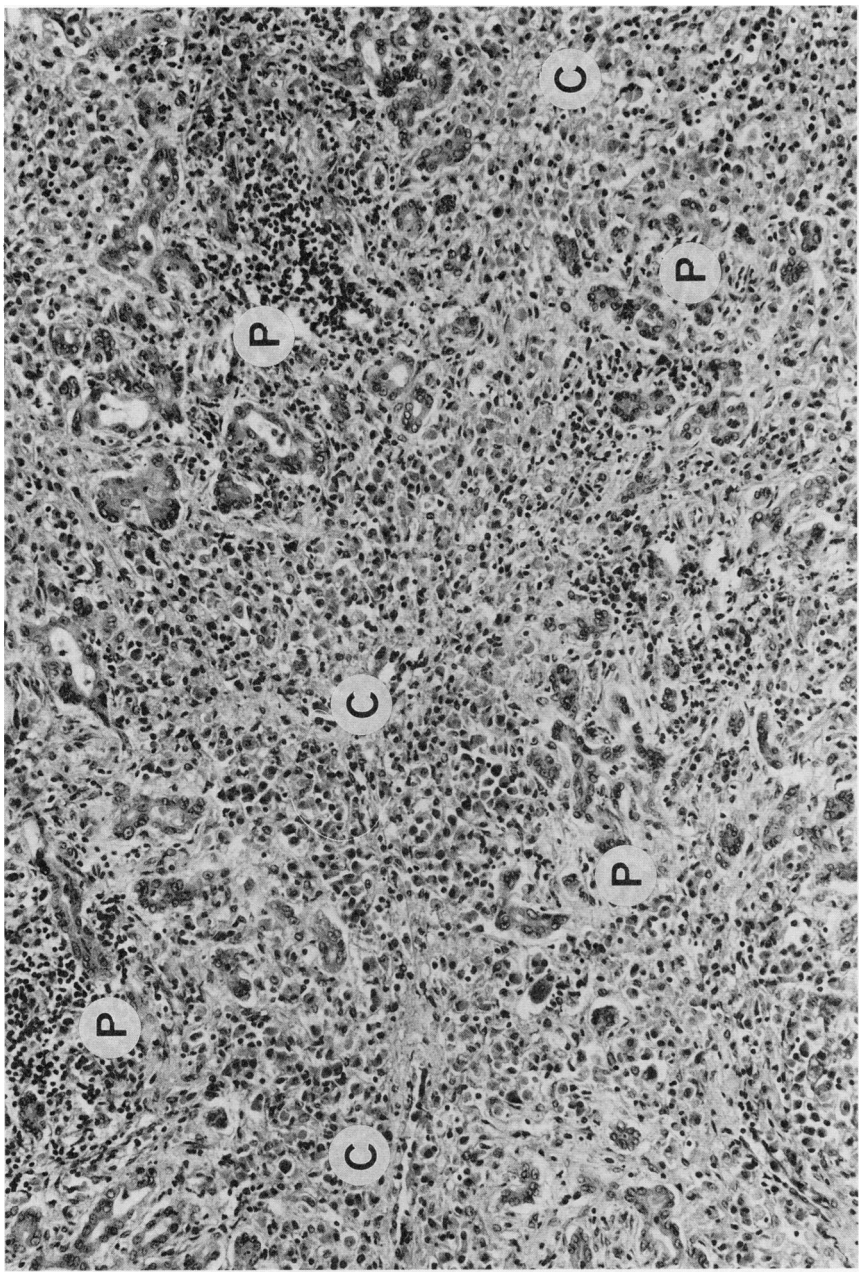

FIG. 9. Multilobular confluent lytic necrosis. Autopsy specimen from patient with fulminant hepatitis B (same case as Fig. 5). Virtually all parenchyma has disappeared in several adjacent lobules; the lobular territories are occupied by some lymphocytes and numerous macrophages. The increased number of ductular profiles around the portal tracts (**P**) is usually interpreted as an attempt at regeneration. **C**: central veins. Hematoxylin and eosin, ×160

necrosis, since it behaves like a membrane-piercing protein [29,30]. Piercing of the cell membrane causes rapid deregulation of ionic pumps, rapid influx of calcium ions, and immediate cell lysis [24].

In summary, the pleomorphic appearance of the liver parenchyma in acute viral hepatitis B seems to reflect a multitude of immunological mechanisms, apparently at work simultaneously or in sequence to eliminate virus-supporting hepatocytes. These mechanisms include: activation of antigen-presenting cells (macrophages), focal apoptosis mediated by infiltrating NK lymphocytes and cytotoxic T lymphocytes, and ballooning of hepatocytes, eventually followed by confluent lytic necrosis, presumably brought about by humoral mechanisms.

The viral antigen(s) which function(s) as target antigen(s) for the immune elimination is (are) not so extensively studied in acute as in chronic hepatitis B; the obvious reason is that diagnostic liver biopsies are usually not taken during the incubation period, but rather at the height of transaminase elevation.

In this fully developed stage of acute hepatitis, only little or no HBsAg or HBcAg can be demonstrated in the liver. This state is consistent with the concept that acute hepatitis B is an "elimination type" of disease, resulting in eradication of the virus and assuring a self-limited course of the disease [8]. The necrotic hepatocytes are phagocytosed by Kupffer cells, which acquire a bulky cytoplasm, loaded with coarse, golden-yellow PAS-positive and diastase-resistant pigment granules termed ceroid pigment. These ceroid-laden macrophages tend to cluster in the parenchyma and finally migrate toward the portal tracts. The loss of parenchymal cells is compensated for by hepatocellular regeneration, which usually leads to complete "restitutio ad integrum" in uncomplicated acute viral hepatitis B.

CHRONIC VIRAL HEPATITIS B

In about 10 percent of patients infected with HBV, the disease which develops corresponds to chronic hepatitis (Fig. 1).

CLASSIFICATION OF CHRONIC HEPATITIS

Histologically, the term chronic hepatitis refers to a broad spectrum of hepatic inflammatory conditions, ranging from near-normal liver histology to complete cirrhotic transformation of the liver architecture. Classification remains a problem, because of the multiplicity of simultaneous lesions, the imprecise borderline between subgroups, the subjective evaluation of individual tissue alterations, the problem of sampling error, and terminologic confusion.

In 1968, an international Liver Study Group proposed a very simple classification of chronic hepatitis [31]. Distinction was made between chronic persistent hepatitis, a milder form of disease with better prognosis, and chronic aggressive hepatitis, a more severe category of disease with frequent evolution into cirrhosis. This classification emphasized piecemeal necrosis (see below) as the important histological hallmark of progressive forms of chronic hepatitis.

In contrast, Dr. Gerald Klatskin and his colleagues from Yale maintained that the real pacemaker for cirrhogenic evolution is bridging hepatic necrosis [32–35]. This controversy became the subject of a long debate. Patterns and extent of piecemeal necrosis and bridging hepatic necrosis were examined in several studies which also intended to evaluate the use of steroid treatment.

These studies were evaluated in a recent editorial in *Hepatology* [36], ending with the main conclusion that in fact the problem is not finally settled: larger and better

stratified series of patients need to be studied. Nevertheless, a unifying picture seems to emerge in that both piecemeal necrosis and bridging hepatic necrosis are prognostically important lesions. The concept that lobular necro-inflammatory lesions, especially more extensive bridging necrosis, characterize more severe variants of the disease, was incorporated in further classifications of chronic hepatitis [37–39].

As a result of ongoing studies, recent schemes of chronic hepatitis have become more complicated but still distinguish between milder forms and more severe variants of the disease (Table 1) [40].

The prototype form of mild chronic viral hepatitis is chronic persistent hepatitis. Histologically, this condition corresponds to "portal" hepatitis [41]. The portal tracts are infiltrated by lymphoid cells, which remain confined within the limits of the portal connective tissue. The lobular parenchyma shows minimal lesions: occasional focal necrosis and rare apoptotic bodies.

The more severe category of chronic hepatitis (chronic active or aggressive hepatitis) comprises several variants with different degrees of liver damage, all characterized by piecemeal necrosis (Table 1).

In mild to moderate chronic aggressive hepatitis, the predominant lesion is periportal piecemeal necrosis; the lobular parenchyma shows only minor changes, including some scattered foci of focal necrosis. This type of chronic hepatitis, although mild, may slowly progress into cirrhosis [42].

More severe variants of chronic aggressive hepatitis show a more complex histologic picture; not only is piecemeal necrosis more extensive, but in addition the lobular lesions are impressive and may include bridging hepatic necrosis (Table 1).

Bridging hepatic necrosis obviously entails more extensive and more rapid dissection of the lobular architecture. The most severe form of confluent lytic necrosis, i.e., multilobular necrosis, may be an especially ominous feature [43]. Dr. G. Klatskin and his colleagues deserve the credit for having emphasized the fundamental importance of bridging hepatic necrosis in accelerating the evolution of chronic hepatitis into cirrhosis [32–35].

Collapse of the reticulin framework in areas of extensive confluent necrosis leads to the formation of fibrous scars; these can be considered as "passive" septa, since they derive primarily from passive collapse of a denudated frame of fibers with subsequent new collagen formation [25]. Passive septa show an excess of fibers over cells; they are paucicellular septa, carrying little inflammatory infiltration, and remain sharply delineated from the surrounding parenchyma. This appearance is in contrast to extending wedges of piecemeal necrosis, rich in cells and poorly delineated, which have been termed active septa.

NATURAL HISTORY OF CHRONIC VIRAL HEPATITIS B

For several years, classification of chronic hepatitis has been the decision base for starting treatment with steroids and/or immunosuppressives. This procedure is no longer the case for chronic viral hepatitis type B [44].

Histological definition of the degree of disease activity and the stage of progression of the disease remains, however, important in the light of newer insights into the natural history of the disease.

From several studies [5,45,46] it appeared that three phases can be recognized over the years during the long course of chronic hepatitis B.

In the first phase, which can be considered as a period of "immune tolerance" to the HBV, extensive viral replication takes place; this process is reflected in positive

TABLE 1
Classification of Chronic Hepatitis

Category	Interstitium	Parenchyma	Architecture
CLH	Mild portal hepatitis	Marked spotty necrosis	Normal
CPH	Portal hepatitis	Spotty necrosis	Normal
Minimal		Minimal	Minimal	
Marked		Marked	Marked	
Chronic septal hepatitis	Mild portal hepatitis	Minimal spotty necrosis	Passive septa—no nodules
NSRH	Mild (variable) portal hepatitis	Occasional spotty necrosis Kupffer cell activation	Normal
CAH	PMN		
Minimal		Minimal PMN	Mild spotty necrosis	Normal
Moderate		Moderate PMN	Marked spotty necrosis	± Disturbed
Severe		Severe PMN active septa	Severe spotty necrosis Focal confluent necrosis Zonal confluent necrosis	Disturbed
Very severe		Severe PMN active septa	BIIN —PC CN —Panlobular CN	Severely disturbed
		Bile duct lesion	—MLN	
Cirrhosis				
Active	PMN	CN	Nodules + active septa
Inactive	Portal hepatitis	Spotty necrosis	Nodules + passive septa

Abbreviations: CLH: chronic lobular hepatitis CPH: chronic persistent hepatitis BHN: bridging hepatic necrosis CAH: chronic active (aggressive) hepatitis PMN: piecemeal necrosis CN: confluent necrosis NSRH: nonspecific reactive hepatitis PC: portal-central MLN: multilobular necrosis

serological reactions for HBeAg, hepatitis B viral DNA, and DNA polymerase activity. Biochemically, the disease is mild, without marked elevation of transaminase levels. Histologically, the liver biopsy reveals only low inflammatory activity, corresponding to chronic persistent hepatitis or non-specific reactive hepatitis.

This form of chronic persistent hepatitis is of the so-called HBcAg-predominance type [47]: the majority of the hepatocytes are positively stained for HBcAg, mainly in their nuclei, and HBsAg is often found in a membranous localization in the liver cell membranes. Nuclei which are overloaded with HBcAg show on hematoxylin-eosin stained sections a peculiar appearance, described as "sanded nuclei" [48]. This stage represents the high-replicative phase of chronic hepatitis.

After some time, and for reasons unknown, the immune system succeeds in eliminating substantial numbers of virus-infected cells. This phase can be considered the "immune-clearance" phase of the disease, characterized by low levels of viral replication and in many patients by seroconversion from HBeAg to anti-HBe antibody. This phase is preceded by or associated with a flare-up of transaminases and is characterized histologically by more impressive parenchymal lesions, recognizable as chronic lobular or chronic active hepatitis. The episode of parenchymal necrosis may be quite severe, with multiple areas of focal necrosis and even bridging hepatic necrosis [49]. Such a necrotizing episode may turn the liver into cirrhosis.

The ensuing third phase is the "non-replicative" phase: one finds no more signs of viral replication and the patient is anti-HBe antibody-positive. Transaminases are only slightly elevated, and, accordingly, liver histology reveals only mild inflammatory lesions of chronic persistent hepatitis. This form of chronic persistent hepatitis is, however, of the so-called HBsAg-predominance type [47]: large numbers of parenchymal cells display a massive accumulation of HBsAg in the cytoplasm, and only few or no nuclei are positive for HBcAg. Such parenchymal cells, containing large amounts of HBsAg in their endoplasmic reticulum, represent the so-called ground-glass hepatocytes [50]. It should be emphasized, however, that the ground-glass appearance of hepatocytes may also be caused by other mechanisms [51].

Apparently, several virus-infected cells have escaped immune elimination. This condition might be due to defective display of viral antigens at the liver cell surface, inadequate display of HLA class I antigens, or masking of membrane-expressed viral antigen(s) by antibody (Fig. 2).

Several studies [52,53] indicate that the latter mechanism is operating in chronic hepatitis B: HBcAg-positive liver cells are covered with immunoglobulin IgG which could be shown to have anti-HBc antibody specificity. This condition adds to the complexity of phenomena occurring in the liver in HBV infection and is part of a complex network of immunomodulation (Fig. 2).

NECRO-INFLAMMATORY LESIONS IN CHRONIC ACTIVE VIRAL HEPATITIS B

Piecemeal Necrosis

Piecemeal necrosis is not unlike interphase dermatitis, observed, for example, in lichen ruber planus or phototoxic dermatitis [54]. In these conditions, mononuclear inflammatory cells concentrate at the interphase between epidermal keratinocytes and dermal connective tissue. The infiltrate is predominantly composed of helper T lymphocytes in the derma, whereas cytotoxic T cells infiltrate between the epidermal keratinocytes, which undergo individual acidophil necrosis (so-called Civatte bodies).

The number of epidermal, HLA class II antigen-positive Langerhans cells is increased, whereas the keratinocytes themselves show increased expression of HLA class I antigens [55] and de novo display of HLA class II antigens [56]. The whole process causes an indistinct borderline between epidermis and derma.

Interphase dermatitis represents a cell-mediated immunological reaction, associated with destruction of epidermal keratinocytes. The antigen against which the reaction is mounted remains unknown.

Piecemeal necrosis in the liver can also be described as an interphase hepatitis [57]. Here also the epithelial-mesenchymal borderline is blurred, lymphocytes invade the epithelial territories, and some hepatocytes appear as acidophil bodies (Fig. 10).

The predominant type of lymphocyte in the invading fronts of piecemeal necrosis is the OKT8+ suppressor/cytotoxic T lymphocyte, whereas OKT4+ helper/inducer T lymphocytes are more numerous in the central area of the portal connective tissue [58].

Some of the portal and periportal mononuclear cells do not correspond to lymphocytes but represent accessory cells, reticulum cells, or antigen-presenting cells.

One type of reticulum cell shows the ultrastructural features of follicular dendritic cells which are well known in the lymph follicles or so-called B areas of lymphoid tissues like lymph nodes and spleen. They are mainly found in the central parts of portal tracts [58,59].

In the peripheral areas of piecemeal necrosis, another type of reticulum cell can be observed. It has the electron microscopic features of interdigitating reticulum cells, which were often described in the paracortical areas or so-called T zones of lymph nodes [59]. These cells are the homologues of the Langerhans cells in the skin. Dendritic and interdigitating reticulum cells appear to function as antigen-presenting cells, respectively, for B and T lymphocytes [60].

The morphological similarity of these reticulum cells to those observed in lymph nodes and spleen, and their preferential localization in central or peripheral areas of piecemeal necrosis, allow us to consider the central part of the portal tract as the B zone, and the invading periphery as the T zone of piecemeal necrosis [58].

Endothelial cells also play an important role in immunological reactions. One of the crucial features is to influence the exit of lymphocytes from blood to interstitium. In areas of piecemeal necrosis, numerous lymphocytes are crowded together (Fig. 10), suggesting that the capillaries in those areas must be a preferential zone of exit from blood to tissue, not unlike the high-endothelial venules in the paracortical areas of lymph nodes.

Ultrastructural investigation of piecemeal necrosis reveals that in these areas the sinusoidal lining endothelial cells lose their fenestrae, show swelling of their cytoplasm, and accumulate electron-dense granules, assuming morphological features resembling those of the lining cells in high-endothelial venules [61,62]. Such transformed endothelial cells presumably may further change into so-called fibroblastic reticulum cells, which are thought to be involved in collagen production. As such, these phenotypic shifts of endothelial cells may represent a link between inflammation and fibroplasia in piecemeal necrosis [58,61].

Liver parenchymal cells show marked changes in expression of HLA antigens in areas of piecemeal necrosis. Normal hepatocytes do not express HLA-ABC antigens; in areas of piecemeal necrosis, however, liver parenchymal cells do display these antigens at their surface [9]. In the same areas, hyperplastic sinusoidal lining cells,

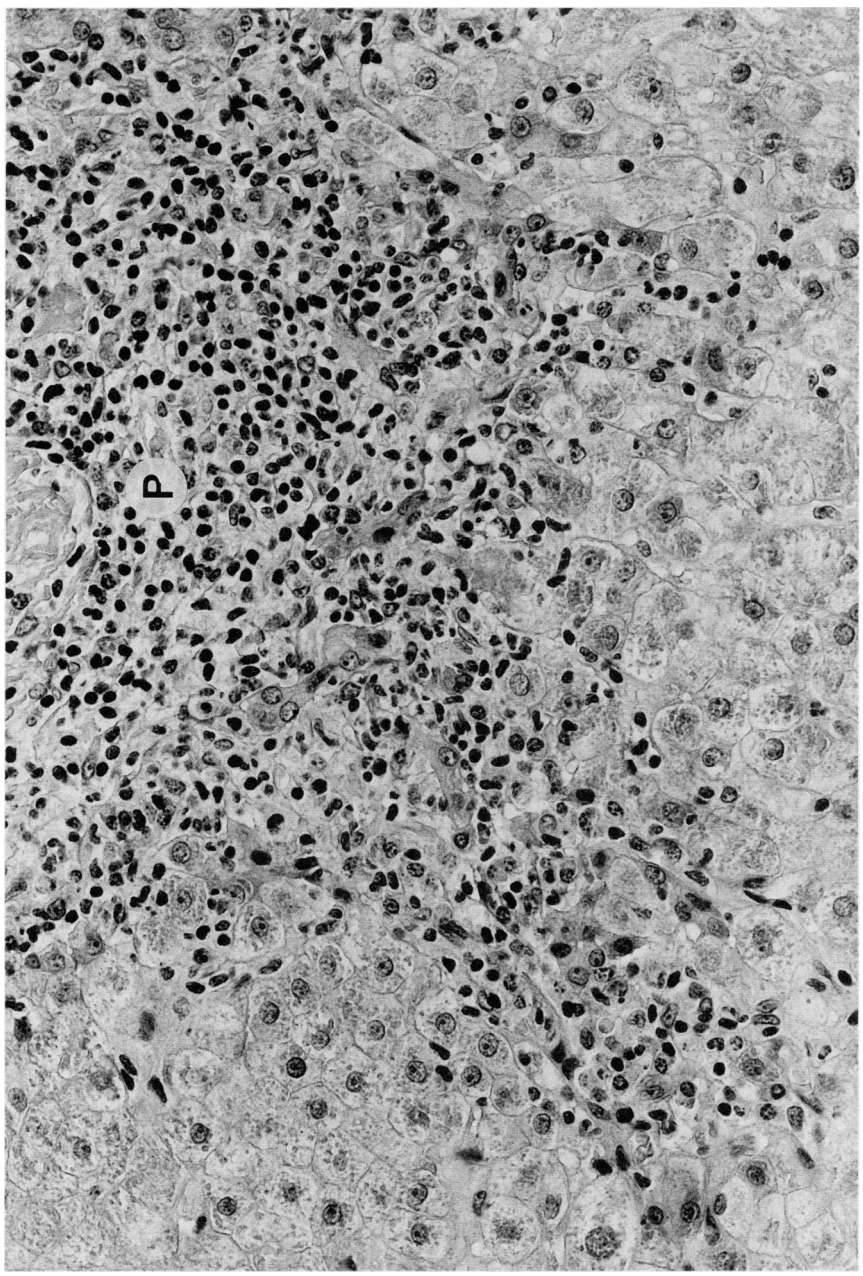

FIG. 10. Piecemeal necrosis in chronic active hepatitis B. The interphase between portal tract (P) and the lobular parenchyma is blurred by infiltration of numerous mononuclear cells, mainly lymphocytes, accompanied by loss of parenchymal cells in the same area. Hematoxylin and eosin, ×400

strongly positive for HLA class II antigens, are found to encircle hepatocytes [9]. The meaning of increased hepatocellular HLA class I antigen expression may be that nearby cytotoxic T lymphocytes have to "see" their target antigen in conjunction with HLA class I antigens; the activation and proliferation of these cytotoxic T cells may be enhanced by increased HLA-DR (class II) antigen expression on the surrounding sinusoidal lining cells [9].

The mode of cell death of the target hepatocytes in piecemeal necrosis appears to correspond to apoptosis [63].

The nature of the target antigen is not known. Apparently, it is not a viral antigen, since neither HBcAg nor HBsAg are preferentially localized in zones of piecemeal necrosis. Some studies suggested that the target antigen may be a self-antigen, termed liver membrane antigen [64]. The latter studies, however, indicate that the effector cells are not cytotoxic T cells, but rather killer cells involved in antibody-dependent cellular cytotoxicity.

It is not impossible that both T-cell cytotoxicity and antibody-dependent killer cell activity occur together in piecemeal necrosis.

Focal Necrosis

Much attention has been focused on the mechanism of elimination of virus-infected cells in chronic hepatitis, causing lobular parenchymal damage and necrosis appearing as focal or spotty necrosis.

Functional immunologic studies [52,65–66] have indicated that in chronic viral hepatitis B, focal necrosis is due to cell-mediated immunity, with cytotoxic T lymphocytes functioning as effector cells against viral nucleocapsid antigen (HBcAg and/or HBeAg) as the target antigen.

Immunohistochemical studies on tissue sections [67] reveal that, in areas of focal necrosis, the infiltrating lymphocytes are OKT8+ suppressor/cytotoxic cells; hepatocytes in these foci display not only HLA class I, but also HLA class II antigens on their membrane, and often contain HBcAg in their nuclei, cytoplasm, and plasma membrane [67]. Immuno-electron microscopy allows us to confirm that HLA class II antigen [67] and HBcAg [68,69] are indeed expressed at the level of the liver cell plasma membrane.

These results are consistent with the conclusions obtained from functional immunological studies that HBcAg is a target antigen, against which effector cytotoxic T cells react when it is expressed together with HLA class I antigens at the liver cell membrane (Fig. 2). The meaning of hepatocellular HLA class II antigen expression is not readily explained, although this expression may allow the hepatocyte to present the viral antigen(s) to T lymphocytes, thus enhancing the immunological response. The "aberrant" expression of HLA class II antigens on epithelial cells has been observed in other tissues as well and indicates that antigen presentation may not be exclusively a function of antigen-presenting reticulum cells [67].

IMMUNOMODULATION IN CHRONIC VIRAL HEPATITIS B

In addition to immunomodulation by anti-HBc antibody, further immunomodulation is brought about by interferons. Alpha interferon from different cellular sources may influence expression of viral antigens and enhance the display of HLA class I antigens [70]. Gamma interferon, produced by sensitized T lymphocytes, may induce enhanced expression of HLA class I and class II antigens on hepatocytes [12,67].

Further components in immunomodulation are immunoregulatory molecules. One such factor is a lipoprotein termed rosette inhibitory factor (RIF) [71]. Incorporation of the HBV genome into infected hepatocytes induces a state of disordered liver cell metabolism, resulting in the induction of abnormal immunoregulatory molecules (including RIF), which are responsible for inhibition of normal suppressor cell function, resulting, in turn, in antibody responses to autologous molecules such as actin, liver specific protein (LSP), and liver membrane antigen (LMA) [72] (Fig. 2). Factors such as Lex (liver extract) [71,73], LIP (liver immunoregulatory protein) [74], and another LIP (liver-derived inhibitory protein) [75] may be responsible for inhibition of cytotoxic effector lymphocytes near necrotizing hepatocytes. Such immune inhibitory effect of factors released from damaged hepatocytes may confine the inflammatory reaction within restricted tissue areas and help to explain the topographical restriction of necro-inflammatory lesions like piecemeal necrosis and focal necrosis in chronic hepatitis B.

CIRRHOSIS

Continuous or relapsing parenchymal damage in chronic hepatitis B is followed by parenchymal regeneration. The development of active and passive septa causes disturbances in the normal spatial relationship between hepatic parenchyma and interstitial connective tissue. Parenchymal regeneration in a nodular fashion, in between the restructured or restructuring fibrous scaffold, leads to progressive development of cirrhosis (Fig. 1).

Post-hepatitic cirrhosis is often of the macronodular variety, characterized by irregular size of the parenchymal nodules and a variable thickness of the fibrous septa.

Even in the cirrhotic stage, the necrotizing and inflammatory process may continue to operate in the already altered and architecturally ruined liver; this situation corresponds to "active" cirrhosis. In liver biopsies from these patients, signs of activity are reflected in the same histological features which also characterize chronic active hepatitis in the non-cirrhotic stage: that is, piecemeal necrosis, spotty necrosis, and confluent necrosis [40].

HEPATOCELLULAR CARCINOMA

Evidence has become compelling that HBV is an important etiological factor in the causation of primary hepatocellular carcinoma [76] (Fig. 1).

Integration of viral DNA into the host DNA of some immune-protected hepatocytes is thought to represent the initiation factor in the sequence of events that finally result in the development of hepatocellular carcinoma [77]. Initially, integration is a random event, with random insertion of random viral DNA segments into cellular DNA. It is followed by gene rearrangements (duplication, deletion, and so on) of viral and host DNA, leading to altered gene expression and cellular transformation [77]. Cycles of cellular necrosis due to the hepatitic process and ensuing regeneration are believed to fulfill the role of promotion [78].

After a long period of selection, a clonal proliferation of cells ensues, endowed with properties of unlimited proliferation and immortality. This stage represents the early stage of malignant growth.

It may occur in non-cirrhotic liver, as is often the case in patients from Africa and the Far East, representing areas with high endemicity of HBV, where infection often

occurs in the perinatal period. In the Western world, hepatocellular carcinoma develops more often on a background of cirrhosis. The tumor cells contain integrated viral DNA but express HBsAg only to a limited extent, and even less HBcAg.

Dysplastic liver cells represent a cellular lesion which has been considered a histological precursor lesion of malignant hepatocytes [79]. Such liver parenchymal cells are mainly characterized by enlargement, irregular size, and hyperchromatic staining of their nuclei. They are frequently observed in HBsAg-positive liver cirrhosis and may occasionally themselves express HBsAg. Whether dysplastic liver cells truly represent a precursor stage of malignant transformation of hepatocytes remains a matter of debate [80].

Early foci of hepatocellular carcinoma may be difficult to diagnose in biopsy specimens from a cirrhotic liver. It would be useful to dispose of reliable markers of malignant hepatocytes. Immunohistochemical staining for hepatic transferrin receptor has been suggested as a useful diagnostic tool [81] which merits further investigation.

CONCLUSION

In conclusion, infection with HBV may lead to a wide spectrum of liver lesions, including diverse cytological alterations, various types and patterns of necrosis, different modes of inflammatory infiltration, normal and aberrant patterns of regeneration and hyperplasia, variable degrees and patterns of fibrosis, including the irreversible stage of liver cirrhosis, and even malignant transformation of hepatocytes.

I had the good fortune to discuss several of these lesions with Dr. Gerald Klatskin on multiple occasions, usually during coffee breaks and social parties at liver meetings. I learned a lot from his distinctive stories and detailed descriptions. Dr. G. Klatskin not only was a leader in clinical hepatology but also a most competent student with encyclopedic knowledge of microscopic liver changes.

With great affection and deep gratitude, I dedicate this review of "liver lesions in hepatitis B virus infection" to the memory of the legendary Dr. Gerald Klatskin.

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