Section 18

LECTURE

Immunology of the Liver

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I. Acute Hepatitis B

Evidence for the role of circulating immune complexes in mediating the extrahepatic features of acute Hepatitis B:

1. "Serum sickness syndrome"

a. Occurs 10-15% in the prodrome usually before jaundice and often confused with other rheumatological diseases. However, HBsAg positive in high titer. SGOT almost always elevated.
b. The syndrome is transient - may persist in some cases as prolonged hepatitis.
c. Characterized by rash (often urticaria), arthralgia, frank arthritis, and angioedema.
d. No residual joint deformity. LE prep, ANA, RF are usually negative.
e. Circulating cryoprotein immune complexes present in high titer during active joint symptoms. Titers rapidly diminish with resolution of joint symptoms.
f. Associated with low serum complement levels (C5, C3, C4), suggesting complement sequence activation.
g. Complement breakdown products (C3a and C3b) detected in serum by immunoelectrophoresis.
h. IgG subtypes in the immune complexes are predominantly complement fixing IgG_1 and IgG_3.
j. Pathophysiology - (probable) viral proliferation in the liver - release of large amounts of HBsAg and ? other antigens into the circulation → early anti-HBsAg response → formation of immune complexes in antigen excess soluble → activation of complement → tissue deposition → tissue injury → rapidly cleared by RE cells as complexes become larger due to continued antibody production → resolution of clinical features. (Note the striking similarity to acute serum sickness produced in rabbits by bovine albumin.)

2. Urticarial rash - Patients may present only with this physical finding as an early clinical manifestation of acute hepatitis B.

a. Also occurs in prodrome of acute hepatitis B infection before jaundice and joint symptoms.
b. Circulating cryoprotein immune complexes detectable in the circulation and composed of the components outlined in Table 1.
c. Serum complement components are depressed during the urticarial rash phase of the illness (see Table 1).
d. Pathology of involved skin demonstrates a necrotizing venulitis characterized by a perivenular infiltrate of polys, macrophages and lymphocytes, fibrin deposition in venular walls, endothelial necrosis, nuclear debris and extravasation of erythrocytes.

e. HBsAg, immunoglobulin and complement are detectable (by immunofluorescence) in the walls of vessels of affected skin but not in uninvolved skin.

f. Immune complexes composed of HBsAg and anti-HBs can be visualized by electron microscopy in cryoproteins isolated from blood

g. Pathophysiology probably similar to patients with the "serum sickness-like" syndrome

TABLE 1: Composition of Immune Complexes

				Cryoprecipitate							Whole Serum			
	Clinical				Complement			Immunoglobulin						
	Day	SGOT (IU)	TB (mg%)	Protein	C3	C4	C5	HBsAg G	α-HBs M	HBsAg A	α-HBs titer	titer	titer	titer
Patient 1:														
Angioedema →		1	1,300	12.9	4+	2+	2+	2+	2+	2+	2+	1:32	1:16	1:1,024 0
Rash →		3	--	--	3+	2+	+	2+	2+	2+	2+	1:64	1:4	1:1,024 0
Arthritis →	9	800	4.3	2+	2+	0	2+	1+	2+	1+	1:512	0	1:256	0
	14	210	2.7	+	1+	0	0	0	0	0	0	0	0	0
	23	100	1.9	+	1+	0	0	0	0	0	0	0	0	0
	31	42	1.3	+	1+	0	0	0	0	0	0	0	0	0
	44	--	--	ND	0	0	0	0	0	0	0	0	0	0
	109	35	1.0	ND	0	0	0	0	0	0	0	0	0	0
Patient 2:														
Arthritis →	1	1,600	2.4	2+	2+	2+	+	+	+	0	0	1:8	0	1:8
	9	800	6.5	2+	2+	2+	+	+	+	+	0	1:4	0	1:8
	16	100	3.1	ND	0	0	0	0	+	0	0	1:8	--	--
	28	56	1.2	ND	0	0	0	0	0	0	0	0	0	1:16
Patient 3:														
Rash →		1	800	1.6	+	+	+	+	2+	2+	+	1:4,096	0	1:1,024 0
Arthritis →	4	400	1.8	3+	+	+	+	2+	2+	+	1:4,096	0	1:256	0
	6	--	--	2+	0	0	0	2+	2+	0	1:4,096	0	1:2,048	0
	8	370	1.1	3+	+	+	+	2+	2+	+	1:1,024	0	1:128	0
	10	--	--	3+	+	+	+	2+	2+	+	1:512	0	1:32	0
	13	370	3.3	+	+	+	+	2+	2+	+	1:512	0	1:128	0
	15	--	--	2+	+	+	+	2+	2+	0	1:128	0	1:64	0
	17	--	--	3+	+	+	2+	2+	2+	+	1:64	0	1:128	0
	22	320	7.3	2+	+	+	2+	+	2+	0	1:2,048	0	1:256	0
	31	1,200	5.8	2+	0	0	0	0	+	0	1:128	0	1:64	0
	39	1,150	7.5	+	0	0	0	0	+	0	1:128	0	1:256	0
	45	380	5.9	ND	0	0	0	0	0	0	1:64	0	0	0
	54	47	2.5	ND	0	0	0	0	0	0	0	1:8	0	0
	60	30	1.3	ND	0	0	0	0	+	0	0	1:16	0	0
	120	25	1.0	ND	0	0	0	0	0	0	0	0	0	1:8

*Cryoprecipitable protein concentration was calculated as the amount of protein measured in the cryoprecipitate resolubilized in 0.5 ml of BSA-containing buffer minus the amount of protein measured in 0.5 ml of the same BSA-containing buffer when no cryoprecipitate was detectable. 4+, 15-20 mg cryoprecipitable protein/0.5 mg; 3+, 10-15 mg/0.5 ml; 2+, 5-10 mg/0.5 ml; +, 1-5 mg/0.5 ml; ND = <1.0 mg/0.5 ml. HBsAg was measured by hemagglutination and hemagglutination inhibition.

3. Vasculitis involving medium size arteries (periarteritis nodosa)

This is a severe systemic disease often leading to death within several months.

a. Syndrome of fever, myalgia, arthralgias, weight loss and abdominal pain. Disease is confined primarily to a necrotizing vasculitis of medium-sized arteries leading to aneurysm and, in some cases, rupture and ischemic tissue injury.
b. 30-40% of patients with the classic features of periarteritis nodosa are infected with Hepatitis B virus.
c. Infarction of the liver may occur due to obliteration of the vascular supply and, in some patients, a progressive, destructive inflammatory picture develops as a result of chronic B virus infection while, in others, minimal liver injury is evident.
d. HBsAg, IgG and C_3 have been localized in walls of affected arteries by immunofluorescent techniques.
e. The pathophysiology is incompletely understood. It has been suggested that, in HBsAg positive patients, immune complexes mediate the vasculitis. However, the physical characteristics of the immune complexes such as size, composition, Ag-Ab ratios and the propensity to localize to medium-sized arteries are unknown.

4. **Proliferative glomerulonephritis** - a rare disease

a. Found primarily in chronic carriers of Hepatitis B virus
b. HBsAg, IgG and C_3 have been detected in glomerular basement membrane by immunofluorescent techniques. Furthermore, these components can be eluted from the kidney.
c. Although it is likely that the renal damage is the result of immune complex deposition, further studies are required.

5. **Essential cryoglobulinemia**

a. A disease of previously unknown etiology characterized by the clinical trial of purpura, arthralgia and weakness frequently accompanied by renal involvement that can be rapidly progressive. Histologic examination reveals widespread vasculitis with immune complex deposition. An important role for the mixed cryoglobulins in the pathogenesis of the immune complex vasculitis has been suggested by the deposits of IgG, IgM and complement, at the site of disease and low serum complement levels.
b. Anti-HBs and HBsAg have been detected in the isolated cryoglobulins of 74% of such patients. Furthermore, HBsAg and intact hepatitis B virus have been observed in the cryoglobulins by electron microscopy. Finally, HBsAg and anti-HBs appear concentrated in the cryoprotein relative to the serum concentration.
c. These observations suggest that some patients with the syndrome of essential mixed cryoglobulinemia where liver disease is not unusual, develop the disease as a result of exposure to hepatitis B.

II. Primary Biliary Cirrhosis

A. Features: This disease is thought to involve destruction of intralobular bile ducts by an immune mediated process. Evidence suggests that cytotoxic T-cells sensitized against biliary antigens may be important. In addition, there is a striking humoral immune response to mitochondrial antigens. Indeed, more than 95% of patients with PBC will have circulating anti-mitochondrial antibodies. As the disease progresses, the late lesions are typified by a paucity of bile ducts (presumably destroyed by the primary process), prominent cholestasis, and fibrosis and cirrhosis. At this stage of the disease, the histology is difficult to distinguish from extrahepatic biliary obstruction and other forms of end stage liver disease which result in cirrhosis (e.g. chronic active hepatitis).

1) Symptoms (non-specific)

a. Insidious - onset over several years
b. Puritus is striking (90%)
c. Fatigue, anorexia, right upper quadrant discomfort
d. Diarrhea
e. Dark urine (due to billirubin) and light stools (biliary obstruction)
f. Ascites and edema (late and due to parenchymal liver cell failure and portal hypertension)

2) Physical Findings

1. Hepatomegaly is the rule
2. Skin changes - planar xanthomas, xanthalasma
3. Cutanous stigmata of chronic liver disease such as palmar erythema and "spider" angiomatosis (late)
4. Splenomegaly and portal hypertension (late)

3) Laboratory Features

a. Striking increase in alkaline phosphatase (4-10x normal) compared to bilirubin
b. Hepatic synthetic function usually well-preserved (albumin, prothrombin time)
c. Circulating "autoantibodies" (see below)
d. Elevated serum IgM levels
e. Increased serum concentration of cholesterol and phospholipids

4) Differential Diagnosis

a. Extrahepatic biliary obstruction, common duct stone, stricture or carcinoma of the pancreas
b. Chronic active hepatitis
c. Drug hepatitis (especially phenothiazines and estrogens)
d. Sclerosing cholangitis

5) Diagnosis

a. Female - pruritus, associated diseases

b. Xanthoma and xanthelasma
c. Striking elevated serum alkaline phosphatase values
d. Elevated IgM levels, circulating "autoantibodies", increased serum concentrations of phospholipids and cholesterol
e. Exclude extrahepatic obstruction and drugs
f. Liver biopsy

B. Associated or Co-existing other Diseases

1) *Systemic sclerosis (scleroderma)*; approximately 10% develop PBC

2) *CREST syndrome* - rare and characterized by the following clinical features:
   a. Calcinosis cutis
   b. Raynaud's phenomenon
   c. Sclerodactyl
   d. Telangiectasis
3) *Sjogren's syndrome*; approximately 20-30% develop PBC
4) *Renal tubular acidosis*; approximately 40-50% develop PBC
5) *Rheumatoid arthritis*; approximately 10-15% develop PBC

C. Immunologic Aspects: There is increasing evidence that immunologic mechanisms are important in the pathogenesis of PBC.

1) General Features

   a. Nature of the histopathologic lesions - intense mononuclear cell infiltration of the portal tracts in association with intralobular bile duct rupture and destruction
   b. Association with other diseases where immunologic mechanism appears important in pathogenesis (e.g., rheumatoid arthritis, Sjogren's syndrome, etc.)
   c. Variety of circulating "autoantibodies"

      i. Anti-mitochondrial (AMA); not species or disease specific, present in high titer; approximate frequency >95%. Recently, AMA were found to react specifically with the pyruvate dehydrogenase complex family of proteins. Four AMA antigens of 74, 50, 50 and 52 kD have recently been described.
      ii. Anti-bile ductular and canalicular; approximate frequency >60%
      iii Anti-smooth muscle; approximate frequency 40-70%
      iv. Anti-nuclear; approximate frequency 20-30%
      v. Rheumatoid factor is an antibody which has specificity for IgG (19S or 7-8S IgM, 7S IgG and even IgA; approximate frequency 15-40%

2) Altered Complement Metabolism

   a. Increased catabolism of C3 and $C1_q$ compared to albumin in PBC. Not found in other liver diseases such as chronic active hepatitis and alcoholic induced liver injury.

b. Increased serum levels of complement components breakdown product: $C3_b$, $C3_d$ and C3 activator fragment (Bb) of C3 proactivator (C3PA) or properdin factor B of the alternate complement pathway (mechanism of activation may be primary or through the amplification loop of the classical pathway).

III. Chronic Active Hepatitis (CAH)

1) Definition. Chronic active hepatitis is a disorder characterized by continuing hepatic necrosis, active inflammation in the liver and progressive fibrosis which may lead to or be accompanied by cirrhosis. This disease has also been referred to as "autoimmune" hepatitis, lupoid hepatitis, plasma cell hepatitis, subacute hepatitis, acute juvenile cirrhosis, and chronic liver disease in young women. Multiple etiologic agents may initiate chronic active hepatitis. Probably the most important etiologic factor is infection with hepatitis B virus. Persistence of hepatitis B surface antigen following acute viral hepatitis has been associated with the development of chronic active hepatitis. In fact, 20-30% of patients with chronic active hepatitis have evidence of active hepatitis B infection. There is an increased incidence of chronic active hepatitis following non-A, non-B post- transfusion hepatitis. Thus, infection by non-A, non-B agents, especially in immunosuppressed renal transplant recipients, may be important in the pathogenesis of some cases of chronic active hepatitis. Drugs such as oxyphenacitin, alpha-methyldopa, and isoniazid have been incriminated in some cases of chronic active hepatitis; however, in the majority of patients, particularly women, the etiologic agent(s) have yet to be defined.

TABLE 2: Comparisons of Cryoprotein Concentration, Serum Complement Levels and Alternate-Complement-Pathway Activation (ACPA) in Eight Patients with Primary Biliary Cirrhosis with the Highest Serum Titer of Immune Complexes

Case	Cryoprotein Conc. (mg/dl)		Serum Complement (mg/dl)		ACPA	Raji Cell Serum Titer (µg/ml)
	IgG	IgM	C3	C4		
1	0	0	180	38	+	2,192
2	2.3	44	196	76	+	1,890
3	0.3	8.4	170	21	--	1,760
4	0	13.4	142	24	--	1,379
5	0	8.9	150	24	--	403
6	0	0.9	168	36	--	370
7	0	10.2	200	9	--	279
8	18.6	22.0	140	29	--	181

2) Pathologic Features. In CAH the cardinal histopathologic features observed in the liver include: 1. a dense mononuclear and plasma cell infiltration of the portal zones which greatly expands these areas with extension of the inflammatory infiltrate into the liver lobule (so-called "piecemeal necrosis") with erosion of the limiting plate.

3) Evidence of hepatic regeneration with "rosette" formation and regenerative

pseudolobules. There is usually evidence of ongoing hepatocellular necrosis with the presence of Councilman-like bodies representing degenerating liver cells. There is substantial morphologic evidence that chronic active hepatitis over a period of months to years will progress, if untreated, to post-necrotic cirrhosis.

4) Symptoms (non-specific)

   a. Fatigue, anorexia and malaise
   b. Recurrent low grade fever
   c. Extrahepatic features may dominate the clinical presentation (see below)
   d. Right upper quadrant discomfort

5) Physical Findings

   a. Hepatomegaly, icterus
   b. Features of extrahepatic involvement (see below)

   c. Cutaneous stigmata of chronic liver disease
      i. palmar erythema
      ii. spider angiomata
      iii gynechomastia
      iv. petechial hemmorhages
      v. Dupuytren's contractures
   d. Ascites, edema (late manifestations)

6) Laboratory Findings

   a. Elevations in alkaline phosphatase, bilirubin, SGOT and SGPT values (variable)
   b. Hypergammaglobulinemia - common (more frequent in women than men)
   c. Low albumin and prolonged prothrombin time (>3 sec) occur late in the disease

7) Differential Diagnosis

   a. May resemble acute viral hepatitis early in the course of the disease
   b. Chronic persistent hepatitis - non-progressive "benign" form of chronic hepatitis - requires no therapy - need liver biopsy to distinguish from chronic active hepatitis
   c. Connective tissue disorders such as rheumatoid arthritis and systemic lupus erythematosus (confused with extrahepatic features associated with CAH)
   d. Wilson's Disease - may present with a picture indistinguishable from CAH before neurologic manifestations become apparent
   e. Primary biliary cirrhosis (pruritus, very high levels of alkaline phosphatase, cholesterol and anti-mitochondrial antibodies help distinguish from CAH)

8) Associated or Co-Existing Other Diseases (more common in females and HBsAg negative patients with CAH)

a. Renal tubular acidosis; approximate frequency 40-60%
b. Sicca syndrome; approximate frequency 20-30%
c. Ulcerative colitis; approximate frequency 10-20%
d. Arthritis; approximate frequency 5-10%
e. Rash (macular, papular, acne); rare
f. Pleurisy; rare
g. Myocarditis; rare
h. Glomerulonephritis; rare
i. Autoimmune hemolytic anemia; rare
j. Neutropenia, thrombocytopenia; rare

9) Immunologic Aspects: There are a number of clinical, laboratory and histologic features of CAH which suggest an ongoing immunologic disorder:

a. General Features

1. Intense lymphocyte infiltration into the portal areas with extension into the liver lobule and intimately associated with hepatocyte cytodestruction
2. Increased frequency of HLA haptotype B_{12} and B_8 and, more recently, DRW_4 in HBsAg negative autoimmune features. These data provide some evidence for genetic susceptibility in CAH.
3. Associated with diseases where an ongoing immunologic injury has been postulated (e.g., proliferative glomerulonephritis, autoimmune hemolytic anemia, arthritis, etc.)

b. Variety of serologic abnormalities and auto-antibodies:
   a. Increased serum immunoglobulin (IgG, IgM and IgA); approximate frequency 50-70%
   b. Smooth muscle antibodies; approximate frequency 40-80%
   c. Anti-nuclear antibodies; approximate frequency 20-50%
   d. Anti-mitochondrial antibodies; approximate frequency 10-20%
   e. L.E. cells; approximate frequency 10-20%
   f. Rheumatoid factor; approximate frequency 10-20%
   g. Heterophile reaction; approximate frequency 5-10%
   h. Anti-gastric anti-thyroid anti-adrenal antibodies; rare
   i. False positive syphilis test; rare

c. Evidence for Circulating Immune Complexes

1. Circulating cryoglobulins - composed of primarily IgG and IgM and C_3 have been found in high concentration during the arthritic phase of illness in some patients. In patients with B virus infection HBsAg and anti-HBs have been identified in such cryoglobulins.
2. In patients with vasculitis and glomerulonephritis immunoglobulin IgG, complement component C_3 and, in some cases, HBsAg, have been localized in the affected arteries and along the subepithelial side of the glomerular basement membrane.

3. Serum complement levels (CH50, C3 and C4) have been found to be markedly depressed during arthritis and rash. Depressed levels return to normal with resolution of symptoms. These findings, in association with high concentrations of cryoglobulins, suggest that complement is being consumed. Furthermore, breakdown components of complement such as C_{3b} and C3a (Bb) have been serologically detected during joint symptoms, again suggesting that the complement sequence has been activated by circulating immune complexes.
4. Circulating immune complexes composed of HBsAg-anti-HBs have been directly observed by microscopy.

In summary, there is good evidence for the presence of circulating immune complex mediating some of the extrahepatic features (arthritis, arthralgias, rash, autoimmune hemolytic anemia, thrombocytopenia, neutropenia [? innocent bystander cells], vasculitis and glomerulonephritis) associated with CAH. However, in many patients, the inciting antigen and corresponding antibody response have not been defined.

d. Evidence for Cell-Mediated Immunity

1. CAH has been observed in patients with severe hypogammaglobulinemia
2. Peripheral blood mononuclear cells have been shown to be cytotoxic toward heterologous cell lines and toward autologous liver cells
3. Mechanisms of cytotoxicity (not established)

a. Specific vs. non-specific
b. Nature of the target cells and antigen
    1) liver specific lipoprotein (LSP)
    2) HBsAg
    3) other "exposed" antigens such as HBcAg
c. Effector cells
    1) T-cell mediated
    2) K-cell mediated (ADCC)

Viral Hepatitis

I. Hepatitis B Virus

1) Three morphologic forms: Dane particle (hepatitis B virion), 22nm sphere, and tubules. All three express HBsAg.

2) Hepatitis B surface antigen (HBsAg) and antibody (anti-HBs) [protective] subtypes breed true and are therefore helpful epidemiologic tools. Subtypes do not determine outcome. Subtype determinants include:

   a. a = common determinant.
   b. d/y, w/r = generally mutually exclusive allelic subdeterminants, other less well-defined determinants.
   c. four major subtypes - adw, adr, ayw, ayr.

3) Hepatitis B core antigen (HBcAg)is part of the nucleocapsid. Antibody to HBcAg (anti-HBc) is serologic marker of ongoing and very recent infection (high titer, IgM class) or of infection in the remote past (low titer, IgG class).

4) An endogenous DNA polymerase and a predominantly double-stranded DNA genome are located within the nucleocapsid. HBV has been classified as an Hepadnavirus, along with similar viruses in woodchucks, Beechey ground squirrels, and Peking ducks. All such viruses have a smilar genomic organization, produce chronic hepatitis and, in the woodchuck, predispose to the development of hepatocellular carcinoma.

5) Hepatitis B e antigen (HBeAg) and antibody (anti-HBe):

   a. HBeAg correlates with ongoing viral replication
   b. Anti-HBe appears serologically following disappearance of HBeAg
   c. HBeAg is a truncated product of HBcAg

A. HBV Mutants

Since HBV replicates through a reverse transcriptase mechanism, there is a high mutation rate compared to other DNA viruses. Several mutations in the viral structural genes have been recently defined that have relevance to clinical disease:

a. Point mutations in the polymerase gene region as well as in promoter enhancer regions have been found in latent forms of the virus that reside in the liver following serological recovery. Thus, there is experimental as well as clinical evidence that in some individuals the virus remains latent in the liver for decades.

b. Two point mutations have been described in the core region that lead to the formation of a stop codon. In such individuals, no e antigen is produced, and they

are characterized by anti-HBe positivity and high levels of replicating virus. This HBV variant has been associated with a more severe form of chronic active hepatitis and cirrhosis as well as fulminant hepatitis B.

c. In the Mediteranean, an HBV variant has been defined where a point mutation has lead to an amino acid change in the adominant epitope in the surface antigen region. This strain has emerged in individuals who have been vaccinated with the recombinant HBV vaccine. These escape mutants may have broad implications for vaccine development.

B. Molecular Aspects

1) Genomic size 3.2 kb - codes for four polypeptides, namely HBsAg, HBcAg, DNA polymerase and X-protein. X-protein - functions as a transactivator protein of other cellular genes and may be important in hepatocyte transformation to hepatocellular carcinoma. This occurs when HBV has integrated into the hepatocyte cellular DNA.

2) Two viral RNA transcripts, 3.5 and 2.5 kb.

3) Two direct repeats - similar to retroviruses.

4) Behaves as non-acute type retrovirus in that integration into host cellular DNA is followed by a long latency before malignant transformation of hepatocytes.

5) Lymphotropic - appears as replicative episomal forms.

6) Latent viral forms exist.

C. Epidemiology

Global immensity of HBV problem: 0.1% of general population in North America, and 5-20% of population in certain Far Eastern and African countries are chronically infected with HBV. 200 million carriers worldwide comprise 5% of world population. Transmission is characterized by:

1) *Percutaneous transmission:* blood products, self-injection, scarification, contaminated needles, hemodialysis, transplant, dental work, tattoo, ear-piercing, acupuncture, arthropod borne.

2) *Nonpercutaneous (or covert percutaneous) transmission:* HBsAg-positive body fluids, oral (inefficient), intimate contact (especially sexual), perinatal (mother-offspring).

3) *Enhanced susceptibility:* immunosuppression, institutionalization, male homosexuality, sexual promiscuity, frequent transfusions (especially of plasma concentrates), health professions.

D. Potential Outcomes

1) Resolution and recovery
2) Fulminant hepatitis
3) Subacute hepatitis leading to cirrhosis
4) Chronic persistent hepatitis
5) Chronic active hepatitis
6) Asymptomatic carrier

E. Chronic Infection

1) Association between life-long HBsAg carriage after infection during childhood and carcinoma of the liver. Seen especially in the Far East and Africa, where the relative risk of liver cancer is more than 200 times higher in HBsAg carriers than in non-carriers.

2) HBV-DNA is integrated into the genome of hepatocellular carcinoma tissue.

3) Some individuals who are chronic carriers with high levels of HBV DNA and chronic active hepatitis will respond to α-interferon therapy (15%) characterized by clearance of virus from the liver. There is also evidence that the diseased liver will improve over time. Limitations include the expense of therapy ($6,000 per year), and that the drug has to be administered parenterally and most individuals who have acquired the infection at birth do not respond.

4) Woodchuck model: infection with woodchuck hepatitis virus (a virus similar in many ways to HBV, both of which have been classified as hepadnaviruses) is associated with hepatitis and hepatocellular carcinoma.

II. delta Hepatitis

a. *Delta agent:* A defective RNA agent which requires coinfection with HBV (or other hepadnaviruses) for its replication.

b. *Structure:* Delta particles measure approximately 37 nm, are double shelled, have delta-expressing nucleocapsid cores and HBsAg on the outer surface.

c. *Epidemiology:* Endemic in some parts of the world (northern Italy, Venezuelan Indians). In non-endemic areas, is observed in population groups whose behavior amplifies its spread: drug addicts, hemophiliacs, homosexuals. Epidemiologically analogous with AIDS.

d. *Clinical importance:* Associated with more severe forms of hepatitis B (chronic hepatitis, fulminant hepatitis). Infection of an asymptomatic carrier with delta may lead to severe chronic hepatitis.

III. Hepatitis A Virus

1) *Detection of hepatitis A virus (HAV) particles in stool and liver:* Early fecal shedding of HAV - peaks prior to onset of clinically recognized hepatitis.

2) *Detection of antibody to HAV (anti-HAV) in serum:* Pattern of antibody (anti-HAV) development: IgM during acute illness, IgG during late convalescence. Fecal IgA anti-HAV (coproantibody) during acute illness.

3) *Diagnosis:*

   a. Fecal excretion of HAV (early) - impractical.
   b. Fecal IgA anti-HAV - impractical, insensitive.
   c. Serum antibody response (1) Paired acute and convalescent serum sample or (2) IgM anti-HAV in acute serum sample (or retrospectively for several months after acute illness) - method of choice.

4) *Characterization:*

   a. RNA virus, classified as an enterovirus (but more heat-resistant)
   b. One serotype worldwide
   c. Has been cultivated *in vitro*

5) *Epidemiology and clinical features:*

   a. Mode of spread: fecal-oral. (1) Not transmitted by percutaneous inoculation in nature. (2) Role of day-care centers in spread of HAV recently appreciated.

   b. Variables which determine frequency of exposure include age, socio-economic class, geographic locale, and institutionalization. Approximately 40% to 60% of urban adults have anti-HAV in serum.

   c. High subclinical attack rate

   d. No chronic carriers

   e. Little, if any, role in chronic diseases of liver

   f. Rare cause of fulminant hepatitis, but generally an uncomplicated illness

IV. Hepatitis C Virus

1. *Virology:*

   a. Belongs to the Flavivirus group (dengue, yellow fever, etc.).

   b. Single-stranded RNA virus of approximately 10,000 bases. Organization: 5' end, followed by 3 structural genes followed by 5 non-structural open reading frames.

   c. Level of replication in liver is low but virus is detected in serum and liver of individuals with acute chronic liver disease by the polymerase chain reaction.

2. *Serology:*

   a. Commercially available assay for easy detection of anti-HCV antibodies of the IgG class.

   b. In chronic infection, levels of antibody are quite high.

   c. In acute infection, levels of antibody are generally low and may become detectable only after weeks or months following acute infection.

   d. Anti-HCV antibody tests have many false positives particularly at low titers.

3. *Epidemiology:*

   a. It is a major cause of post-transfusion hepatitis (60-70%).

   b. Significant cause of chronic hepatitis and cirrhosis not related to HBV infection.

   c. Particularly prevalent in Japan and associated with hepatocellular carcinoma.

   d. Hemophiliacs

   e. Percutaneous inoculation, occupational contact, institutional and familial spread.

   f. Similar to epidemiology of type B hepatitis

4) *Clinical Features:* Similar to Type B Hepatitis

   a. Mean incubation period after transfusion approximately 50 days. (Another series of hepatitis cases has a shorter incubation period of 1 to 4 weeks and probably represents another distinct viral strain called hepatitis E virus.)

   b. Less severe than type B hepatitis during acute illness.

   c. Progression to chronic liver disease in approximately 40 to 50% of transfusion-associated cases (<10% in non-transfusion settings).

   d. Probable asymptomatic chronic carrier-state (potentially as many as 3-7% of population).

V. Prevention

A. Type A Hepatitis

1. Immune globulin: more likely to prevent infection than to attenuate illness, i.e., passive-active immunity less likely than anticipated from earlier observations.

a. Post-exposure

1. Household contacts
2. Institutional contacts
3. Dose 0.02 ml/kg

b. Pre-exposure

1. Travel to endemic areas

a. <3 months: 0.02 ml/kg
b. >3 months: 0.05 ml/kg Q4-6 months

2. Hepatitis Type B - See lecture on hepatitis B vaccine

3. Hepatitus C Virus

c. Transfusion-associated: Eliminate commercial donors

B. Hepatitus C Virus

a. Screen for anti-HCV antibodies. Evidence indicates that there is a chronic carrier state associated with high titer anti-HCV. Such individuals show viral genome by the polymerase chain reaction. Thus, in HCV infection, the presence of antibody does not necessarily mindicate serologic recovery from infection and indeed, represents ongoing active replication much like HIV. Major problems include many false negative and false positive results. Second and third generation tests using peptides derived from structural genes are in development.

b. Transfusion-associated - Eliminate commercial donors

c. Screening for anti-HBc reduces post-transfusion hepatitis by 20-30%

d. Screen blood donors for ALT (SGPT) and eliminate donor units with high ALT (at an ALT cut-off of 45, 30% of post-transfusion hepatitis cases could be prevented)

C. Hepatitic B Infections

1) Passive Immunoprophylaxis: hepatitis B immune globulin (HBIG) containing high-titer anti-HBs.

a. Current recommendations: Post-exposure prophylaxis for acute, intense exposure

a. Needlestick, oral ingestion of HBsAg-positive material, i.e., mucosal penetration (the only FDA-approved recommendation for HBIG).

b. Spouse of patient with acute type B hepatitis

c. Newborn of mother with acute type B hepatitis during third trimester or of chronic HBsAg carrier mother. Early administration, preferably in the delivery room, is crucial; repeat at 3 and 6 months. Reduces incidence of HBsAg carriage by 75%.

b. Dose: 0.06 ml/kg (administer a second dose one month after first). For newborn, 0.5 ml at birth, then same dose at three and six months.

c. Transfusion-associated type B hepatitis (massive exposure)

d. Prophylactic administration less effective than eliminating HBsAg-positive and commercially obtained donor blood. 90% of transfusion-associated hepatitis is non-B.

e. Efficacy of globulin preparations in such massive exposure has not been determined, but HBIG, 0.06 ml/kg, immediately after exposure and repeated one month later, has been recommended.

2) Hepatitis B Vaccine: Plasma derived (22 nm purified HBsAg particles) or Recombinant (cloned HBsAg gene expressed in yeast). Hepatitis B vaccine is expensive, approximately $150 for a full course of three injections, and is limited in supply. Moreover, to avoid the cost of unnecessary vaccination of persons who are already immune, prevaccination serologic screening, also costly, must be considered. Still, despite its high cost, the vaccine has been shown to be cost-effective for groups at risk of becoming infected with HBV. Vaccination strategies include:

a. Populations with a <u>low</u> baseline prevalence of immunity but <u>high</u> attack rate of new infections such as new health care workers: Vaccinate without screening - the costs of universal screening in this case would not be justified by the small number of immune persons identified who would be spared the costs of vaccination.

b. Populations with a high prevalence of immunity and <u>high</u> attack rate such as homosexual males: Screen everyone, vaccinate only susceptible.

c. Populations with low prevalence of immunity and <u>low</u> attack rates

3) Indications for Vaccination

a. Pre-exposure vaccination is recommended for groups at risk. These include health care workers exposed to blood (e.g., hemodialysis staff, surgeons, laboratory and blood bank personnel), residents and staff of custodial institutions for the developmentally handicapped, male homosexuals, users of illicit intravenous drugs, patients with hereditary hemoglobinopathies and clotting disorders who require long-term therapy with blood products, household and sexual contacts of HBV carriers, hemodialysis patients, young children living in areas where HBV is endemic, and prisoners.

b. Post-exposure vaccination is indicated in combination with HBIG, for those exposed percutaneously to HBV, sexual contacts of patients with acute HBV infection, and newborns of HBsAg-positive mothers.

c. Alternative types of vaccine are being considered. These later-generation vaccines may be less costly and more available.

a. Polypeptide
b. Recombinant DNA
c. Synthetic polypeptide vaccine

VI. Chronic Hepatitis

A. Etiologies

1) Chronic Hepatitis B Hnfection

a. More common in males
b. Usually not associated with "autoimmune" diseases and a variety of "autoantibodies" such as AMA, LE cells, anti-smooth muscle antibodies, anti-nuclear antibodies, etc.
c. Relatively resistant to all forms of therapy (see below).

2) Drug-Induced Hepatitis (examples): INH, Oxyphenacitin, Aldomet

3) "Autoimmune" Hepatitis

a. More common in females
b. Associated with other "autoimmune" diseases such as renal tubular acidosis, ulcerative colitis, arthritis, etc.
c. Abnormal serologic reactions with hypergammaglobulinema, anti-mitochondria, anti-nuclear, anti-smooth muscle, anti-gastric, anti-adrenal antibodies. Positive LE cells.
d. Responds well to prednisone and imuran therapy.

4) Post-Transfusion Hepatitis

a. "Hepatitis C" viral agents implicated.
b. Distressingly high incidence of progression to cirrhosis.
c. Serologic abnormalities such as autoantibodies are unusual.
d. Therapy with alpha interferon appears to hold great promise in the treatment of chronic hepatitis due to hepatitis C virus infection. Recent controlled studies suggest that 40% of individuals will have a virologic, serologic, and histologic improvement due to therapy.
e. Increasingly more common as the result of coronary artery bypass surgery and multiple transfusions.

B. Pathology

a. Differentiation between chronic active hepatitis and chronic persistent hepatitis.
b. Progression to fibrosis and post-necrotic cirrhosis.
c. Identification of viral antigens at the tissue level by immunofluorescent and molecular hybridization techniques.

C. Immunopathogenesis

a. Ongoing cytodestructive process in the liver
b. Association with other autoimmune diseases
c. Variety of circulating autoantibodies
d. High level of circulating immunoglobulins
e. *In vitro* cytotoxicity of lymphocyte to autologous and heterologous cell lines
f. Association with HLA haplotypes B1 and B8 (more recently DrW4)
g. Evidence for loss of immunoregulatory control

D. Therapy

1. Chronic active hepatitis B

    a. Prednisone and imuran - generally not effective
    b. Interferon and interferon inducers
    c. Improves viral markers such as HBsAg levels and DNA polymerase while on therapy
    d. Subgroup may benefit - data not clear
    e. Side effects - major problems
    f. Liver disease does not improve in most patients
    g. Problem of HBV viral integration into host hepatocyte DNA
    h. Thymosin - experimental - not proven

2. Drug-induced -- withdraw agent

3. "Autoimmune"

    a. Prednisone and imuran quite effective
    b. Dosage and schedules
    c. Duration of therapy
    d. Symptomatic vs. asymptomatic patients

4. Post-transfusion associated chronic active hepatitis (interferon best therapy available).

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