Virchows Arch [Pathol Anat] (1986) 409:657–667

Virchows Archiv A
© Springer-Verlag 1986

Hepatitis A-like non-A, non-B hepatitis: Light and electron microscopic observations of three cases*

H.P. Dienes[1], Th. Hütteroth[2], L. Bianchi[3], M. Grün[4], and W. Thoenes[1]

[1] Pathologisches Institut der Universität Mainz, Langenbeckstr. 1, D-6500 Mainz, Federal Republic of Germany
[2] I. Medizinische Klinik der Universität Mainz, D-6500 Mainz, Federal Republic of Germany
[3] Pathologisches Institut der Universität Basel, Switzerland
[4] Innere Abt. St. Vincenz Hospital, D-6500 Mainz, Federal Republic of Germany

Summary. To date, three types of NANBH have been distinguished by epidemiological, clinical and experimental data. We examined the liver biopsies of three patients with an acute NANBH resembling hepatitis A from the infection route, incubation period and clinical course. The liver biopsies revealed lesions with a portal and periportal predominance, thus also exhibiting parallels with hepatitis A on the histopathological level.

Key words: Non-A, non-B hepatitis – Hepatitis A – Histopathology of viral hepatitis

Introduction

At present three forms of non-A, non-B hepatitis (NANBH) are distinguished on the basis of epidemiology, clinical observations, and transmission studies (Tabor 1985). Waterborne NANB viruses have been the cause of epidemics in India, an epidemic in Nepal and sporadic cases in India, the Soviet Union, and the Lebanon. This type of NANBH, showing striking similarities with hepatitis A, may also occur in Central Europe, in persons returning from Asia or Africa as has been reported by French authors (Bernuau et al. 1985).

To date, little is known about the histopathology of this type of hepatitis. We report on light and electron microscopic findings as well as on the immunohistological identification of the inflammatory infiltrate in the liver of 3 patients who had acquired this type of NANBH in Africa.

Offprint requests to: H.P. Dienes at the above address

* The work was supported by the Deutsche Forschungsgemeinschaft SFB 311

Case reports

Patient 1. The 36 year-old pregnant woman (29th week) was admitted to our hospital in a state of deep hepatic coma. Prior to admission, she had been living in Sudan for two years in good health. One week before she was transported to Germany, she complained of right upper quadrant pain and malaise. A scleral icterus and the onset of premature labour was noted at that time. Rapid deterioration of health developed and she was delivered of a macerated fetus. Because of imminent liver failure she was transferred to our hospital. On examination the patient was a deeply jaundiced woman with peripheral oedema and hepatic coma II–III. The liver and spleen were not enlarged and lymph nodes were not palpable. The biochemical tests showed elevated transaminase activities (GOT 144 and GPT 218 U/L) and a bilirubin level of 16.6 mg per 100 ml. RIA's for hepatitis B and A viruses assiciated antigens (AUSAB, CORAB, HAVAB, Abbot) revealed low titers of anti-HBs and anti-HAV of IgG type consistent with past hepatitis A and B. All other tests for hepatitis agents such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) were negative. Tropical diseases such as malaria or yellow fever were excluded. Despite intensive care treatment and haemodialysis the patient developed irreversible circulatory failure and died one week later in a hepatic coma.

Patient 2. During the course of her disease the patient's (no. 1) 5 year-old daughter was cared for by an 50 year-old woman, a close friend to the family. After a short period of time, two weeks and a half exactly, the woman experienced fatigue and abdominal pain. On admission, she presented with scleral icterus. The vertical span of the liver was 12 cm and the spleen was not palpable; there was no evidence of ascites. The gallbladder had been removed several years ago. Biochemical tests revealed elevated transaminase (GOT 376 U/L and GPT 637 U/L) and alkaline phosphatase (296 U/L) activities. The total bilirubin was 1.9 mg per 100 ml. Tests for viral hepatitis A and B antigens and antibodies were negative as well as testing for CMV and EBV. The diagnosis of acute hepatitis was assumed on the basis of a liver biopsy (see below) and without any therapy, the patient's health improved within two weeks. A follow-up examination, months later, provided no evidence of chronic liver damage.

Patient 3. This patient was a young man who had returned from a short trip to Africa as a tourist. He was admitted to our hospital because of increasing jaundice and nausea. On examination, he was a 24 year-old sturdy young man with deep jaundice of sclerae and skin. A patchy erythema was found on the extensor surfaces of the extremities. The vertical span of the liver was percussed at 13 cm, the spleen was not palpable. Biochemical tests revealed elevation of transaminase activities (GOT 125 U/L, GPT 789 U/L) and an alkaline phosphatase of 297 U/L. The total bilirubin was 12.5 mg per 100 ml. Anti-HAV antibodies were positive for IgG at a titer of 1:50 (HAVAB) and anti-HAV IgM negative. All HBV associated antigens as well as antibodies were negative when tested by a commercial RIA (AUSRIA, AUSAB, CORAB). CMV and EBV infection were excluded by serological tests. After a transient increase of bilirubin values up to 14.4 mg per 100 ml, the patient was discharged from the hospital although the transaminases were still elevated. A follow-up after six months, however, showed normal biochemical tests and the patient was in excellent health.

Material and methods

In all three cases a percutaneous needle biopsy of the liver was performed, in case 1 immediately after death. The tissue was fixed for light microscopy in 4% neutral buffered formaldehyde, for electron microscopy in 2.5% phosphate buffered glutaraldehyde, and for immunoelectron microscopy in a solution of periodate-lysine-paraformaldehyde, as reported previously (Dienes et al. 1984). For light microscopy the sections were stained with H & E, Prussian Blue, Masson-Trichrome, and periodic acid-Schiff after diastase digestion (DPAS). For electron microscopy, the tissue was embedded in Epon and ultrathin sections were cut on a Reichert microtome. The sections were stained with uranylacetate and lead citrate and viewed with a Philips EM 401.

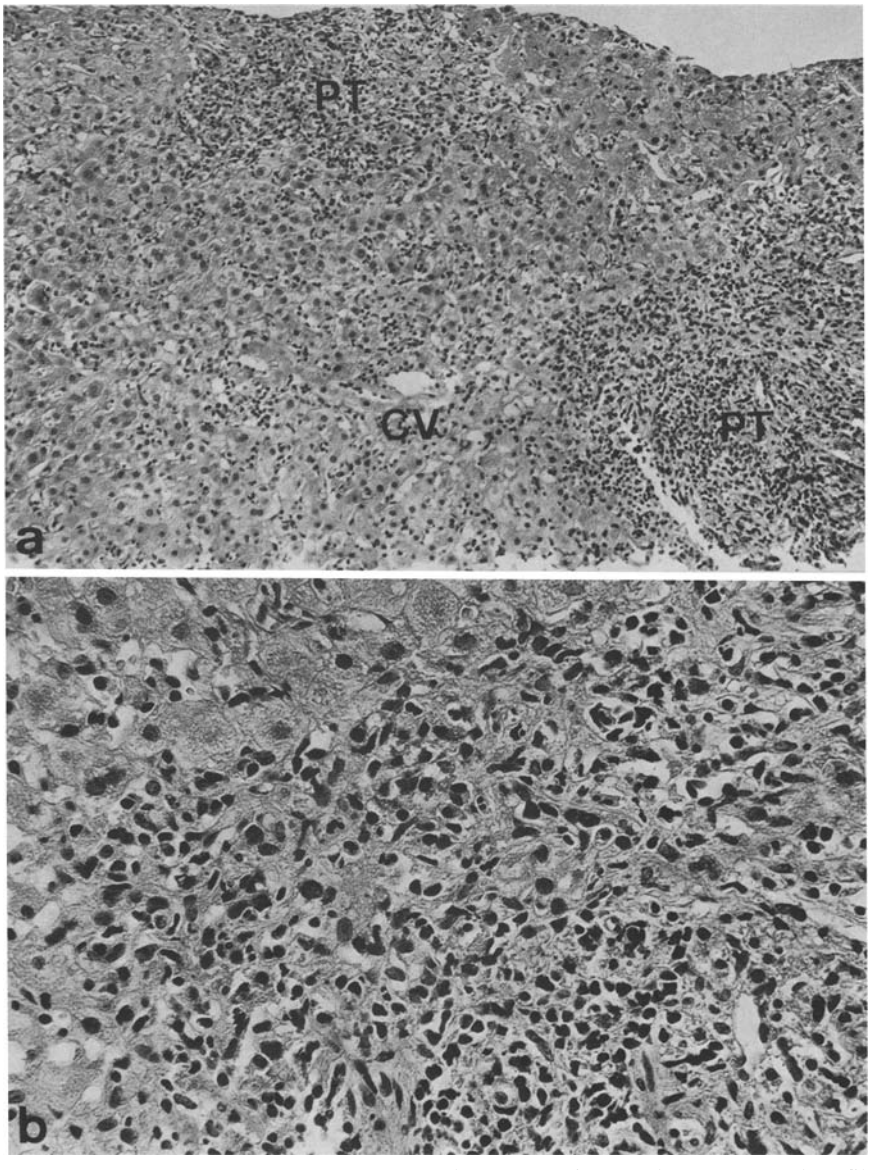

Fig. 1. Liver biopsy from patient 2: **a** the enlarged portal tracts (*PT*) are densely infiltrated by lymphocytes, whereas the lobule (*CV*=central vein) is affected to a relatively mild degree (H & E, ×110); **b** higher magnification of the periportal area with lymphocytes spilling over into the parenchyma (H & E, ×320)

For immunoelectron microscopy after fixation and freezing, the incubation with the following antisera was performed over night on cryostat sections: OKT8 and OKT4 (both from ORTHO), T1B and B1 (Coulter), Leu7 and HLA DR (Becton & Dickinson) and HLA I (Seralab). As the secondary antiserum, goat anti-mouse IgG (DAKO) was used and incubated for 2 h. After applying the disclosure solution with DAB, the sections were embedded in Epon on the slides and further processed in the same way as for routine electron microscopy.

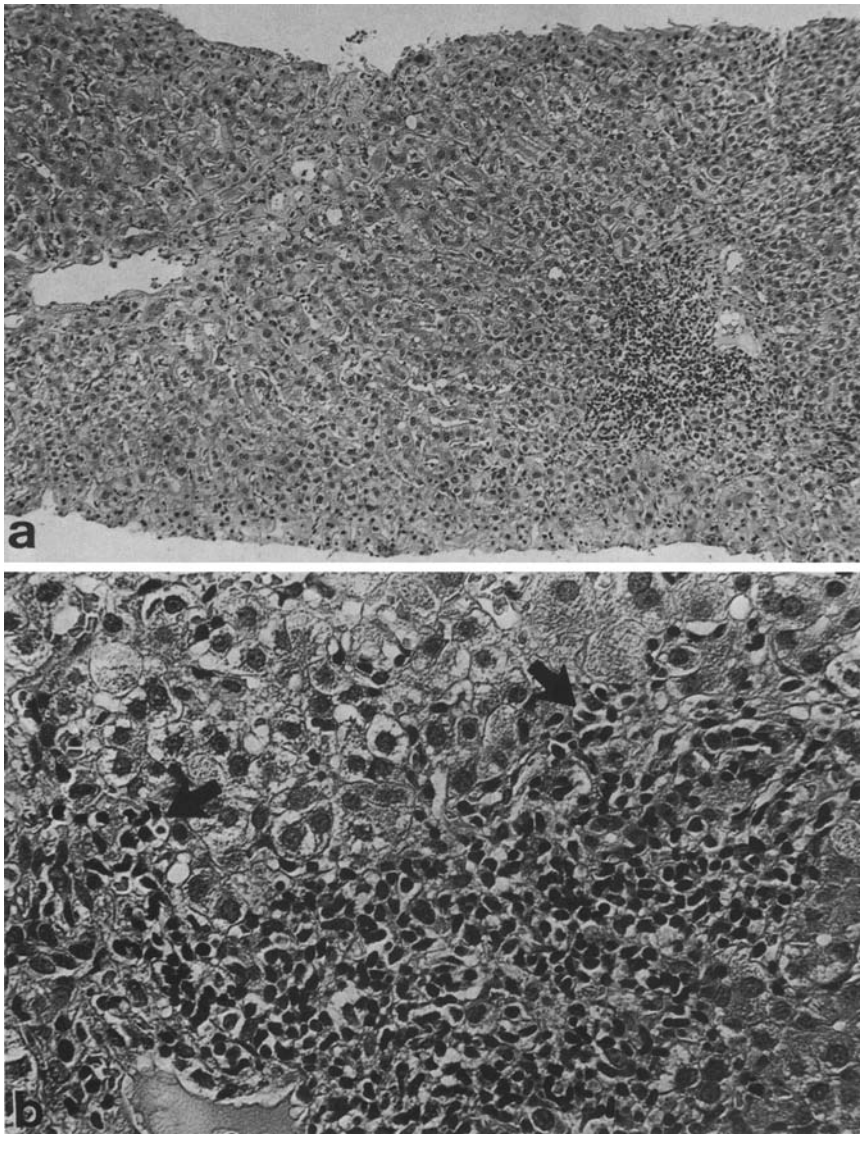

Fig. 2. Liver biopsy from patient 3: **a** inflammatory infiltrate of the portal tract is prominent while the lobule is almost devoid of lymphocytes (H & E, ×110); **b** higher magnification of the portal tract with spillover of lymphocytes into the parenchyma (↑); (H & E, ×420); **c** centrilobular area with cholestasis (↑); (H & E, ×420)

Results

Light microscopy

The liver of patient 1 showed an almost complete loss of the lobular architecture with pseudoglandular transformation of hepatocytes surrounding inspissated bile plugs. Moderate necroinflammatory changes were still present in the preserved parenchyma.

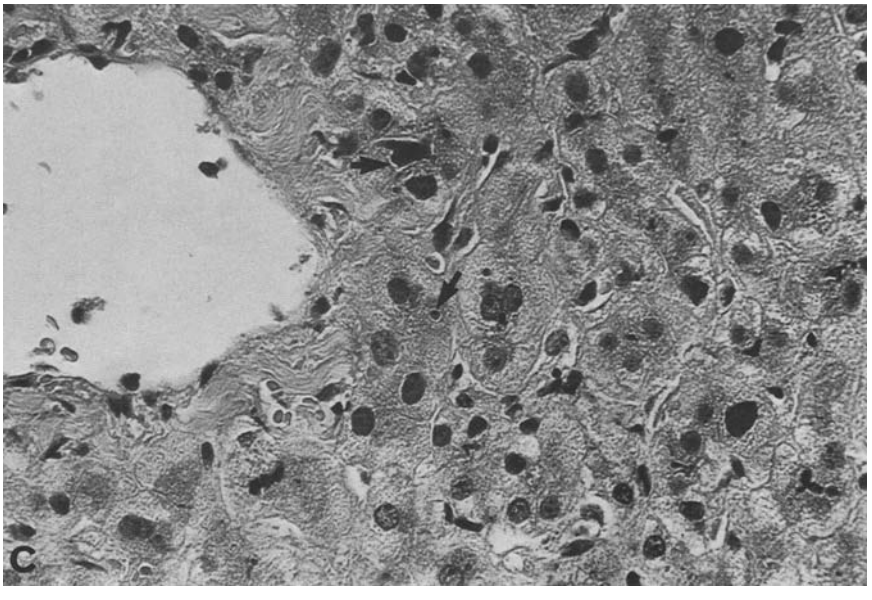

Fig. 2

The liver biopsies of case 2 and 3 displayed enlarged portal tracts (Fig. 1) with a considerable inflammatory infiltrate consisting mainly of lymphocytes (Fig. 2). A spill-over of the inflammatory cells into the neighbouring parenchyma was noted in many areas; genuine piecemeal necroses, however, were not detected. The hepatocellular changes in the lobule consisted of ballooning or eosinophilic shrinking of the heptocytes. A few acidophilic bodies without a significant lymphocytic reaction were scattered throughout the lobule. In the centroacinar zone severe cholestasis was present (Fig. 2c).

Electron microscopy

In patient 1, the liver cells showed severe damage with destruction of cell organelles. Fatty metamorphosis was noted as well as bile accumulation and an increased number of phagolysosomes.

The other two liver biopsies revealed considerable cell polymorphism: Hepatocytes with ballooning degeneration showed vesiculated endoplasmic reticulum. Other hepatocytes displayed shrinking and condensation of the cytoplasm and cell organelles resulting in a web-like pattern. The bile canaliculi were dilated and exhibited a paucity of stub-shaped microvilli.

Virus-like particles were not detected in any of the three biopsies.

Immunoelectron microscopy (IEM)

Case 1. HLA DR was found on all Kupffer cells staining the entire membrane. Most of the lymphocytes were also positive. HLA class I antigens

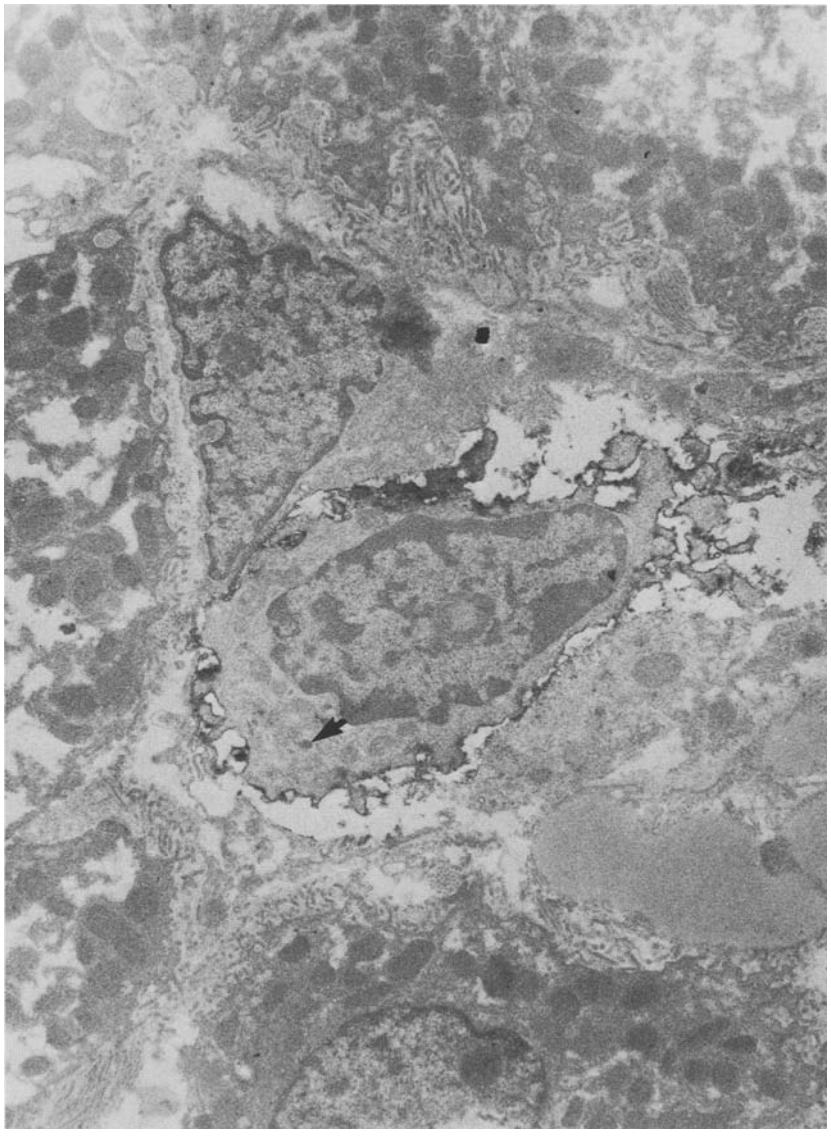

Fig. 3. Patient 3: Leu7-positive NK cell in the sinusoid exhibiting a short uropode and few granules in the cytoplasm (↑) (× 14,800; DAB reaction with uranylacetate counterstain)

were expressed on the hepatocytes including the sinusoidal as well as the lateral membrane and the bile canaliculi. 142 lymphocytes were analysed regarding the membrane markers: 15 were positive for T4, 62 positive for T8 in the lobule and 26 in the portal tracts; 20 lymphocytes exhibited the Leu7 antigen. B1 positive cells constituted only a minority of all lymphocytes (19 were counted).

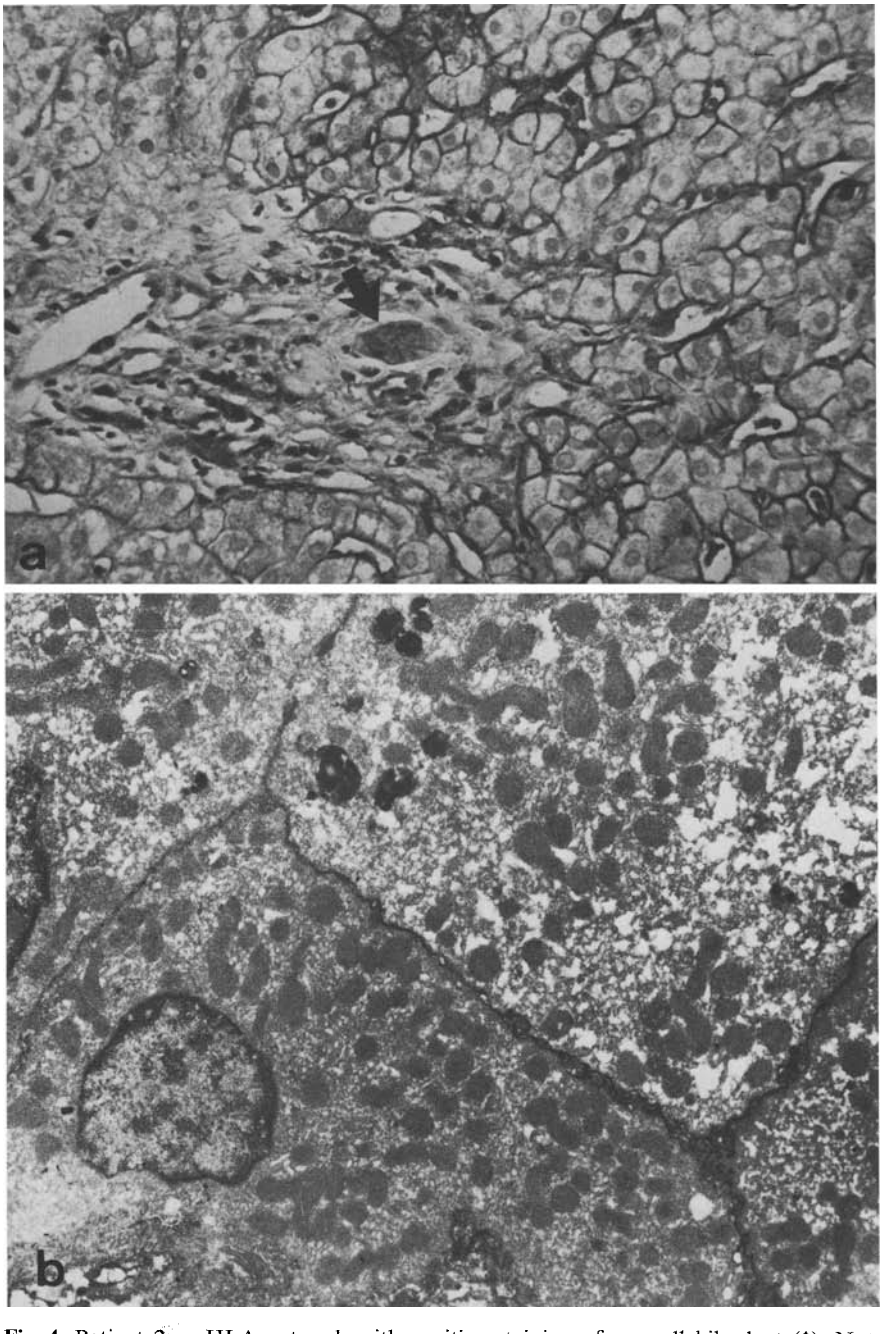

Fig. 4. Patient 2: **a** HLA network with positive staining of a small bile duct (↑). Note the honey combpattern of the parenchyma (Meyer's hemalum, ×380). **b** Same section as Fig. 4 prepared for IEM: the entire circumference of the hepatocytes is stained by anti-HLA antibodies (×5,500)

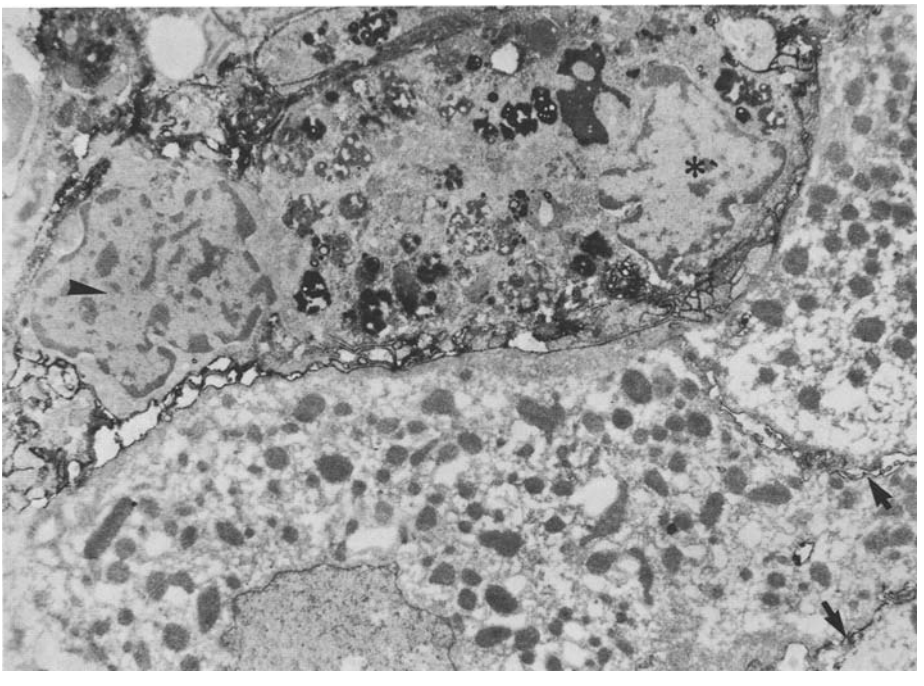

Fig. 5. Patient 2: Staining for HLA DR reveals a strong reaction on the membrane of a Kupffer cell (*) and of a lymphocyte (▲) in close contact to each other. Note a weak binding to the membrane of the hepatocyte as well (↑) (×4,800)

Table 1. Summary of the subtyping of lymphocytes

Type of cell	Number of cells in percent of all lymphocytes		
	pat. 1	pat. 2	pat. 3
T8	62	41	50
T4	11	19	17
Leu7	14	26	17
B1	13	14	16

Case 2. The staining for HLA DR (Fig. 5) and HLA class I antigens (Fig. 4) showed almost an identical pattern with case 1. 185 lymphocytes were counted: 75 were positive for T8, 49 positive for Leu7 and 35 displayed T4 antigens. B1 was positive on 26 lymphocytes.

Case 3. As in the other biopsies, anti HLA DR antibodies bound on all of the Kupffer cells and on the majority of the lymphocytes. HLA I was expressed mostly on the sinusoidal membrane of the hepatocytes, in focal areas on the entire circumference of the membrane resulting in a honeycomb pattern. 189 lymphocytes were subdivided in T and NK lymphocytes: 95 belonged to the T8 subtype, 32 were positive for T4 and the same number

for Leu7. B1 was found on 24 cells. Lymphocytes positive for T4 or T8 could not be distinguished by morphology reliably, although there was the impression that on the whole, the T8 positive cells displayed more cell organelles in a broader cytoplasm. Leu7 positive cells all showed granules in the cytoplasm (Fig. 3) and none of them contained structures with a parallel tubular array.

A summary of the staining results is seen in Table 1.

Discussion

The first serologically documented reports of epidemic hepatitis not caused by HAV were published in 1980. Wong and coworkers (1980) tested serum samples from hepatitis outbreaks in India (Delhi, Ahmedabad, and Pune) and excluded the possibility that these epidemics were caused by HAV. In the same year, Khuroo published the results of his investigations of the waterborne epidemics of viral hepatitis in Kashmir valley (1980). He also found no evidence that the outbreaks were due to HAV infection, although the epidemiology and clinical picture closely resembled hepatitis A. There have been more epidemics; one in India, one in Nepal (Maynard 1984) and a third in Algeria (Belabbes et al. 1984). Reports of sporadic cases of this disease have come so far from the Soviet Union (Balayan et al. 1982) and Lebanon (Shammaá 1984). French authors have diagnosed this type of NANBH in persons returning to France after a recent stay in Africa (Bernuau et al. 1985).

There is ample evidence that in all our patients the hepatitis agent was transmitted by an enteric route similar to that of HAV. In the first patient, the route of the infection remains obscure; but from the fact that the patient's daughter infected a close friend, patient no. 2, within a short span of time (15–17 days), it may be concluded that the first patient bore a contagious agent transmissible by faecal-oral route. Patient no. 3 gave the typical example of a German tourist contracting hepatitis during his vacation in a tropical country. Most of these cases are of the hepatitis A-type (Frösner et al. 1979) but may also be NANBH as our patient demonstrates.

So far no detailed histopathology of this type of hepatitis has been published. The liver lesions of the first patient showed massive necrosis with regenerative changes and are not very specific for the disease. In biopsies 2 and 3, however, characteristic features were found: portal tracts and periportal region were the most affected areas and displayed dense lymphocytic infiltrates. In the centrilobular area, the necroinflammatory changes were only of moderate degree with few acidophilic bodies and a mild activation of Kupffer cells. On the basis of the morphology alone, hepatitis A would have been diagnosed since the lesions resemble those first described by Popper et al. (1980) and Dienstag et al. (1976) in chimpanzees infected with HAV. Although other authors (Kryger and Christoffersen 1982; Okuno et al. 1984) dispute the existence of a distinct histopathological pattern of hepatitis A in humans, some characteristic changes have been evaluated in larger series of biopsies from patients with hepatitis A (Abe et al. 1982;

Korb 1984): Predominance of the inflammatory infiltration in the portal and periportal area with liver cell necroses mimicking piecemeal necroses; sparing of the centrilobular zone which displays only cholestasis.

On the basis of histopathology it seems therefore impossible to distinguish this type of NANBH from hepatitis A. Acute type B hepatitis, however, may develop a different histological pattern with hepatocellular damage and dense inflammatory reaction showing a centrilobular predominance (Bianchi 1983; Dienes et al. 1983; Popper et al. 1980).

Electron microscopy has not been very helpful in establishing the diagnosis. No virus-like particles were found, especially no viruses resembling the recently described 27 nm particles (Kane et al. 1984).

Immunoelectron microscopy revealed some remarkable results: In all 3 biopsies an increased expression of HLA class I-antigens was present as an indicator of inflammatory activity (Thomas et al. 1982). Not only the sinusoidal membrane but also the lateral segment and the bile canaliculi were stained with the antibodies resulting in a honeycomb pattern. A strong HLA DR expression including the entire membrane was noted on all Kupffer cells, which may be interpreted as a sign of activation. The dominant cell population of the inflammatory infiltrate was the T8 positive T cells as has been demonstrated in other tpes of hepatitis by many authors (Eggink et al. 1982; Thomas and Lok 1984). However, in contrast to the biopsies from patients with a chronic course of hepatitis, the percentage of Leu7 positive cells was relatively high. This is in accordance with the observation of Eggink et al. (1984), that in the acute phase of viral hepatitis, NK cells play a more dominant role than in the chronic course of the disease. All of the Leu7 positive lymphocytes showed large granules in the cytoplasm whereas lymphocytes with parallel tubular arrays representing a different type of NK cells (Payne and Glasser 1981) were completely absent.

Acknowledgements. The excellent technical assistance of M. Wörsdorfer is acknowledged. We appreciate the help of C. Bürkner for preparing the manuscript.

References

1. Abe H, Beninger PR, Ikejiri N, Setoyama H, Sata M, Tanikawa K (1982) Light microscopic findings of liver biopsy specimens from patients with hepatitis A and comparison with type B. Gastroenterology 82:938
2. Balayan MS, Agafonov VI, Andzhaparidze AG, Savinskaia SS, Sgibov IA (1982) Viral hepatitis, similar in clinical picture and epidemiology to hepatitis A, but differing from it etiologically. Zh Mikrobiol Epidemiol Immunobiol 8:79–84
3. Belabbes H, Benatallah A, Bougermouth (1984) A Non-A, non-B epidemic viral hepatitis in Algeria. In: Vyas GN, Dienstag JL, Hoofnagle JH (eds) Viral hepatitis and liver disease. Grune & Stratton, New York, p 637
4. Bernuau J, Margulies A, Dubois F, Rueff B, Goudeau A, Benhamou JP (1985) Asian-African non-A, non-B fulminant viral hepatitis in France. J Hepatol (Suppl) 1:21
5. Bianchi L (1983) Liver Biopsy Interpretation in Hepatitis II. Pathol Res Pract 178:180–213
6. Dienes HP, Essling K, John HD (1984) Application of immunoelectron microscopy for identification of T lymphocyte subsets and HLA DR antigen possitive cells in tissue. J Histochem Cytochem 32:547–551

7. Dienes HP, Grün M, Hess G, John HD (1983) Simultaneous infection with non-A, non-B viruses in a patient exhibiting different histologic patterns of acute hepatitis. Liver 3:385–391
8. Dienstag JL, Popper H, Purcell RH (1976) The pathology of viral hepatitis types A and B in chimpanzees. Am J Path 85:131
9. Eggink HF, Houthoff HJ, Huitema S, Gips CH, Poppema S (1982) Cellular and humoral immune reactions in chronic active liver disease. I. Lymphocyte subsets in liver biopsies of patients with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. Clin Exp Immunol 50:17–21
10. Eggink HF, Houthoff HJ, Huitema S, Wolters G, Poppema S, Gips CH (1984) Cellular and humoral immune reactions in chronic active liver disease. II. Lymphocyte subsets and viral antigens in liver biopsies of patients with acute and chronic hepatitis B. Clin Exp Immunol 56:121–128
11. Frösner GG, Papaevangelou G, Bütler R, Iwarson S, Lindholm A, Courouce-Panty A, Haas H, Deinhardt F (1979) Antibody against hepatitis A in seven European countries. Am J Epidem 110:63–69
12. Kane MA, Bradley DW, Shrestha SM et al (1984) Epidemic non-A, non-B hepatitis in Nepal: Recovery of a possible etiologic agent and transmission studies in marmoset. J Am Med Assoc 252:3140–3145
13. Khuroo MS (1980) Study of an epidemic of non-A, non-B hepatitis: possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am J Med 68:818–824
14. Korb G (1984) Die Virus-A-Hepatitis. DMW 109:110–112
15. Kryger P, Christoffersen P (1982) Light microscopic morphology of acute hepatitis non-A, non-B. A comparison with hepatitis type A and B. Liver 2:200
16. Maynard JE (1984) Epidemic Non-A, Non-B Hepatitis. Semin Liver Dis 4:336–339
17. Okuno T, Sano A, Deguchi T, Katsuma Y, Ogasawara T, Okanne T, Takino T (1984) Pathology of acute hepatitis A in humans. Am J Clin Pathol 81:162–169
18. Payne CM, Glasser L (1981) Evaluation of surface markers on normal human lymphocytes containing parallel tubular arrays: A quantitative ultrastructural study. Blood 57:567–571
19. Popper H, Dienstag JL, Feinstone SM, Alter HJ, Purcell RH (1980) The pathology of viral hepatitis in chimpanzees. Virchows Arch (Pathol Anat) 387:91–106
20. Shamma'a M (1984) Acute viral hepatitis in Lebanon: evidence for an HAV-like non-A, non-B hepatitis. Liver 4:39–44
21. Tabor E (1985) The three viruses of non-A, non-B hepatitis. Lancet i:743–746
22. Thomas HC, Lok ASF (1984) The immunopathology of autoimmune and hepatitis B virus-induced chronic hepatitis. Semin Liv Dis 4:36–46
23. Thomas HC, Shipton U, Montano L (1982) The HLA system: Its relevance to the pathogenesis of liver disease. In: Popper H, Schaffner F (eds) Progress in liver disease, vol VII. Grune & Stratton, New York, pp 517
24. Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM (1980) Epidemic and endemic hepatitis in India: Evidence for a non-A, non-B hepatitis virus aetiology. Lancet ii:876–879

Accepted May 13, 1986