Virchows Arch [Pathol Anat] (1985) 408:1–13

Virchows Archiv A

Plasma cells in acute hepatitis: an ultrastructural study

Krzysztof A. Bardadin* and Peter J. Scheuer
Department of Histopathology, Royal Free Hospital and School of Medicine, Pond Street, London NW3 2 QG, United Kingdom

Summary. Plasma cells and their precursors were studied by electron microscopy in liver biopsies from 41 patients with acute viral or drug-induced hepatitis. Mature plasma cells showed the ultrastructural features of the reticular or lymphatic type. Blast cells of different types were also observed. Type 1 predominated in classical acute hepatitis, and appears to transform directly into mature plasma cells. Type 2 corresponds to the centroblast of lymphoid tissue. It was found in fully developed hepatitis, especially when necrosis was severe. Type 3 resembled the centrocyte of lymphoid organs; it was seen particularly in viral hepatitis, and only in severe cases with extensive necrosis. The type 4 plasmablast had the ultrastructural characteristics of a plasmacytoid T cell.

Key words: Hepatitis, viral, human – Plasma cell – Ultrastructure

Introduction

The exact sequence of events and mechanisms of parenchymal damage in acute hepatitis remain uncertain. Predominance in liver tissue of lymphocytes of suppressor/cytotoxic phenotype (Govindarajan et al. 1983; Pape et al. 1983; Shibata et al. 1984) suggests that T cell-mediated cytotoxicity is involved. On the other hand the presence of plasma cells in the inflammatory infiltrate, hypergammaglobulinaemia, circulating immune complexes and specific antibodies against viral components support the participation of humoral immune mechanisms. Plasma cells in hepatitis have been studied by immunocytochemical methods (Paronetto et al. 1962; Hadziyannis et al. 1969), and Mietkiewski and Scheuer (1985) have shown that in acute viral hepatitis IgG- and IgA-containing plasma cells are an important component of the inflammatory infiltrate. Electron microscopical studies have been

* *Present address:* Postgraduate Medical Centre, Warsaw, Poland

Offprint requests to: P.J. Scheuer at the above address

few, and have largely concentrated on the relationship between lymphoid cells and hepatocytes in chronic active hepatitis (Cuccurullo et al. 1976; Kawanishi 1977; Bernuau et al. 1982). The present study is an attempt to characterise the ultrastructural features of plasma cells in acute hepatitis, in the light of recent descriptions of this series of cells in lymphoid tissue and lymphomas (Lukes and Collins 1975; Rilke et al. 1978; Lennert et al. 1983).

The present concept of plasma cell lineage and maturation is that the fully developed, classical plasma cells represent the end stage of the transformation of B lymphocytes, and that their maturation takes place in the germinal centres of lymphoid follicles. On the basis of electron microscopical characteristics, mature plasma cells can be subdivided into reticular (Marschalko) plasma cells on the one hand, and lymphoplasmacytoid or lymphatic plasma cells on the other (Müller-Hermelink and Lennert 1978). There is a further type, the T-associated plasma cell or plasmacytoid T cell, which resembles a plasma cell ultrastructurally but does not appear to produce immunoglobulins (Müller-Hermelink et al. 1973; Müller-Hermelink et al. 1983; Papadimitriou et al. 1983). Its function is not known. In the present study these different types of plasma cells were looked for, and their presence in the different aetiological types and morphological forms of acute hepatitis was documented.

Materials and methods

Forty-one liver biopsies from patients with acute hepatitis were studied. All patients had been investigated at the Royal Free Hospital, London between 1979 and 1984. Biopsies were taken between the second and twentieth week of the illness. Thirteen patients had type A hepatitis, confirmed by presence of IgM anti-HAV in the serum. Seven had type B, and in 6 a diagnosis of non-A, non-B (NANB) hepatitis was made by exclusion of known causes. In the remaining 15 the hepatitis was attributed to drug idiosyncrasy; the drugs involved included halothane, antituberculous drugs and a variety of other agents. Hepatitis was subdivided morphologically (International Group 1977) into classical acute hepatitis (21 biopsies), acute hepatitis with bridging necrosis (11 biopsies), acute hepatitis with panacinar necrosis (4 biopsies) and acute hepatitis with periportal necrosis (5 biopsies). Sinusoidal cell changes in these specimens have been described in detail by Bardadin and Scheuer (1984). The biopsies studied form part of a group the light microscopical aspects of which are described elsewhere (Mietkiewski and Scheuer 1985). Small portions of the biopsy specimens were fixed immediately after the biopsy procedure in 3% glutaraldehyde in cacodylate buffer, dehydrated and embedded in Lemix resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Philips 201 transmission electron microscope. Nearly all plasma cells and likely plasma cell precursors seen in the material were photographed for further study.

Results

Plasma cells and their precursors were found in all biopsies examined, and were only absent from sections with no portal tracts or little inflammatory infiltrate. The number of plasma cells paralleled the severity of the inflammation.

Most of the plasma cells were of the reticular type, with uniform ultrastructural features (Fig. 1). They were oval in shape, sometimes slightly

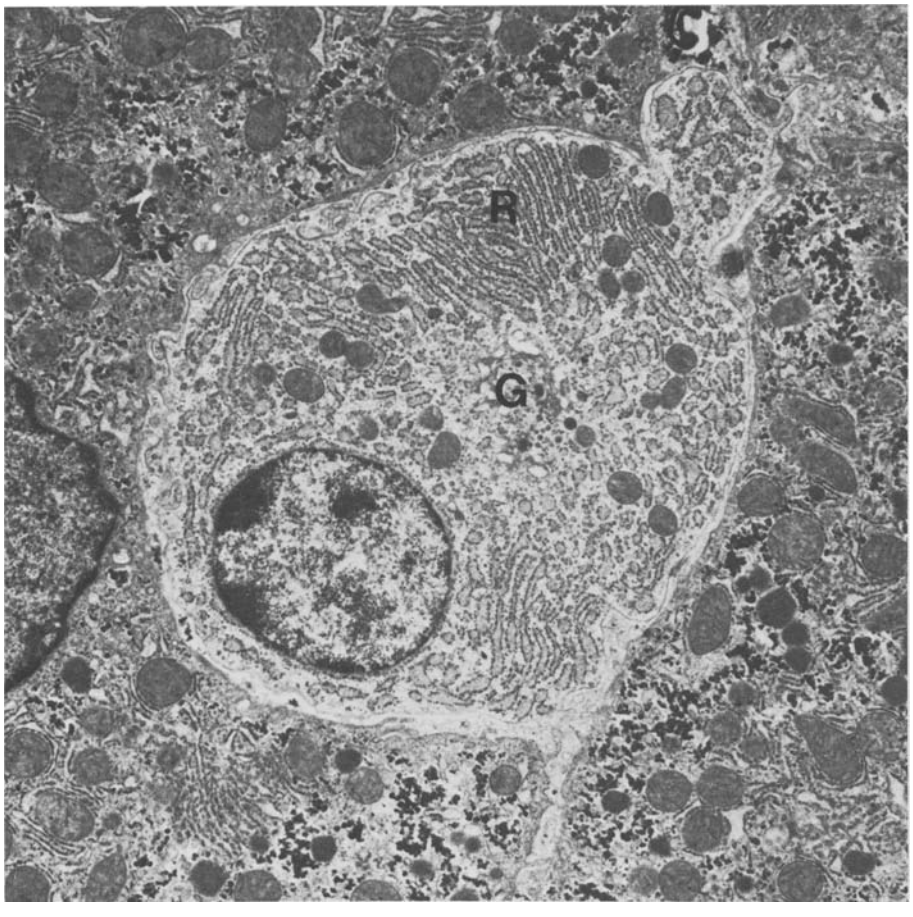


Fig. 1. Reticular plasma cell surrounded by hepatocytes. Note the abundant rough endoplasmic reticulum (*R*) and prominent Golgi apparatus (*G*) containing electron-dense granules. × 6200

irregular in outline, with an eccentric nucleus. The abundant cytoplasm was characterised by parallel strands of rough endoplasmic reticulum filled with amorphous electron-dense material, scanty, usually oval mitochondria, and prominent Golgi apparatus containing electron-dense granules. Many of the plasma cells showed evidence of degeneration including irregular shape, dilatation of the rough endoplasmic reticulum, aggregation of chromatin near the nuclear membrane and apoptosis. Plasma cells of the reticular type were typically seen near capillaries or proliferating bile ductules, or as solitary cells in the space of Disse, in close contact with hepatocytes. Within necrotic areas they were also found extravascularly as part of the inflammatory infiltrate. There was no obvious difference in the number of reticular plasma cells in the various aetiological categories.

In biopsies showing severe acute hepatitis with much necrosis, smaller numbers of plasma cells of somewhat different appearance were found.

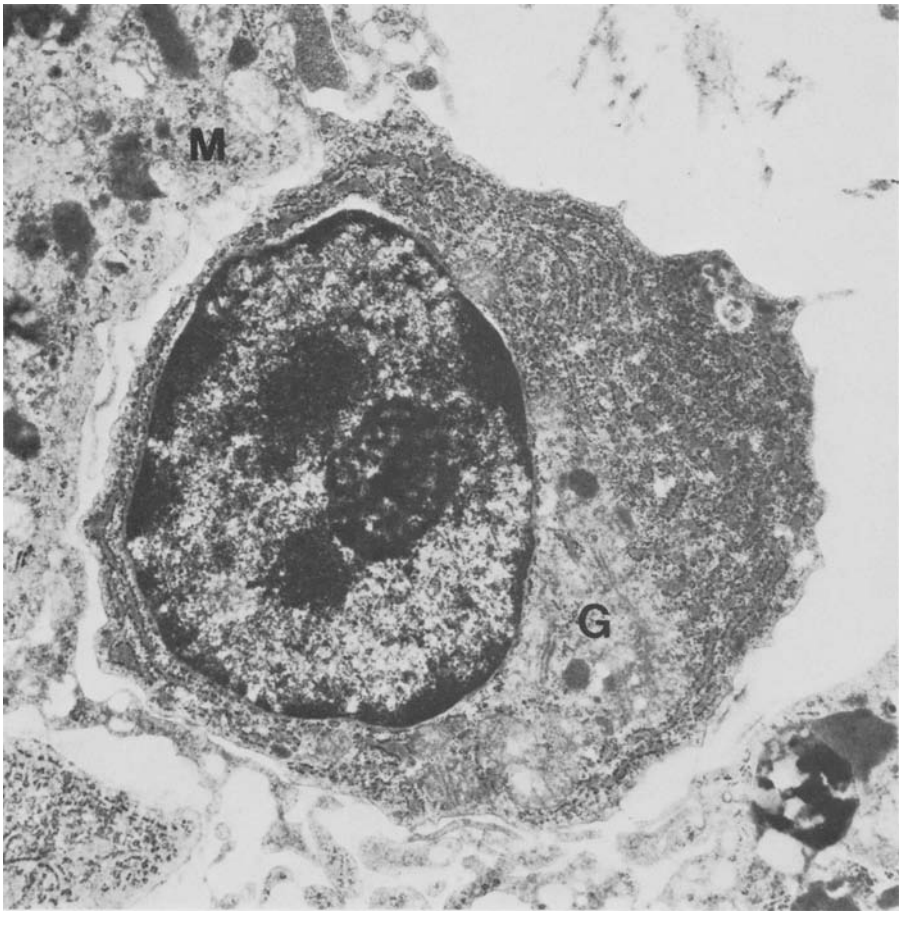


Fig. 2. Plasma cell of lymphatic type, with higher nuclear-cytoplasmic ratio than the reticular plasma cell in Fig. 1. The Golgi apparatus (*G*) is well seen. The cell is in contact with macrophages (*M*). ×11,700

These were smaller and more oval in shape, with a higher nuclear-cytoplasmic ratio (Fig. 2). Other characteristics were as in the reticular type. These smaller cells were classified as lymphoplasmacytoid or lymphatic plasma cells. They were usually located within the sinusoidal lumens.

Whereas the appearances of the above two types of plasma cells were remarkably uniform, much more variation was seen among blast cells showing evidence of plasmacytic differentiation. These blast cells could be divided according to their appearance into four types:

Type 1. These were lymphoblasts with a low nuclear-cytoplasmic ratio and an eccentric nucleus with dispersed chromatin (Fig. 3). A central nucleolus was sometimes seen. The overall diameter of these cells was between 9

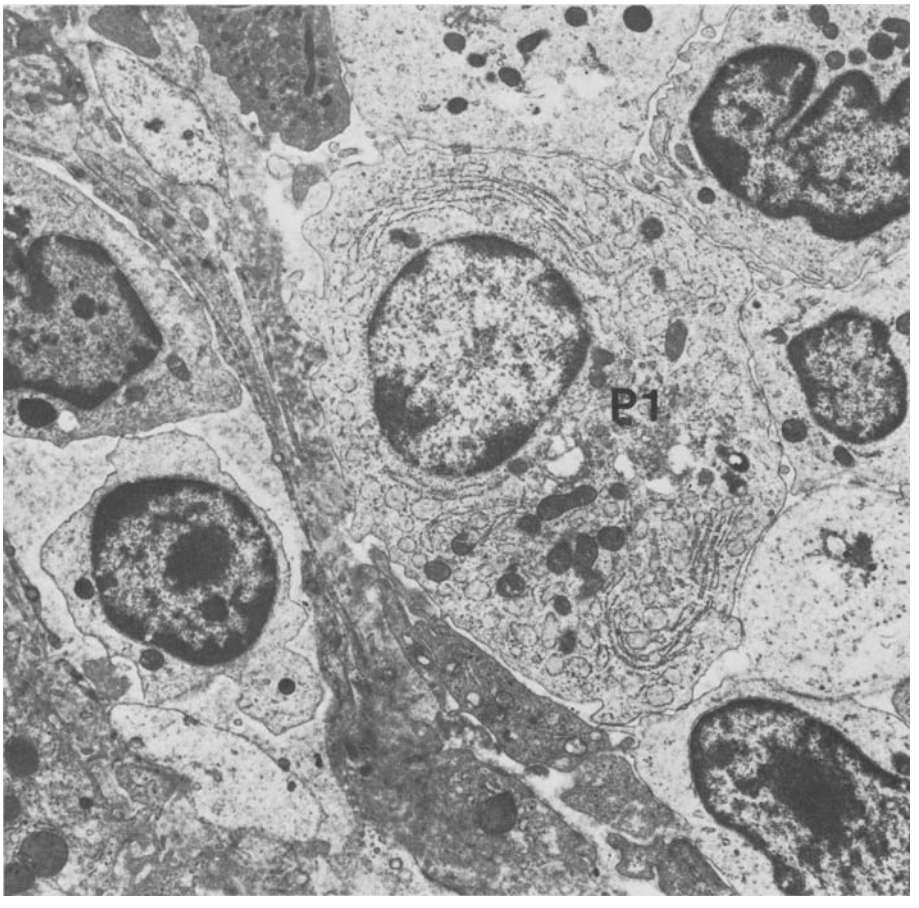


Fig. 3. Type 1 plasmablast (*P1*) within an inflammatory infiltrate. The cell resembles the reticular plasma cell in Fig. 1, but contains much less rough endoplasmic reticulum. ×6200

and 12 μm. The cytoplasm contained well-developed Golgi apparatus, scanty small mitochondria and strands of rough endoplasmic reticulum as well as monoribosomes. Transformation into plasma cells of reticular type appeared to take place by a focal increase of the rough endoplasmic reticulum which radiated from the Golgi apparatus and successively filled the cytoplasm (Fig. 3). Type 1 plasmablasts were mainly seen in the classical form of acute hepatitis, in the first month of the disease.

Type 2. These cells were larger than type 1 (12–20 μm in diameter), with an irregular shape and low nuclear-cytoplasmic ratio (Fig. 4). The large, slightly irregular nucleus contained sparse chromatin and from one to three marginally situated nucleoli. Many monoribosomes, scanty polyribosomes, solitary strands of rough endoplasmic reticulum and oval mitochondria

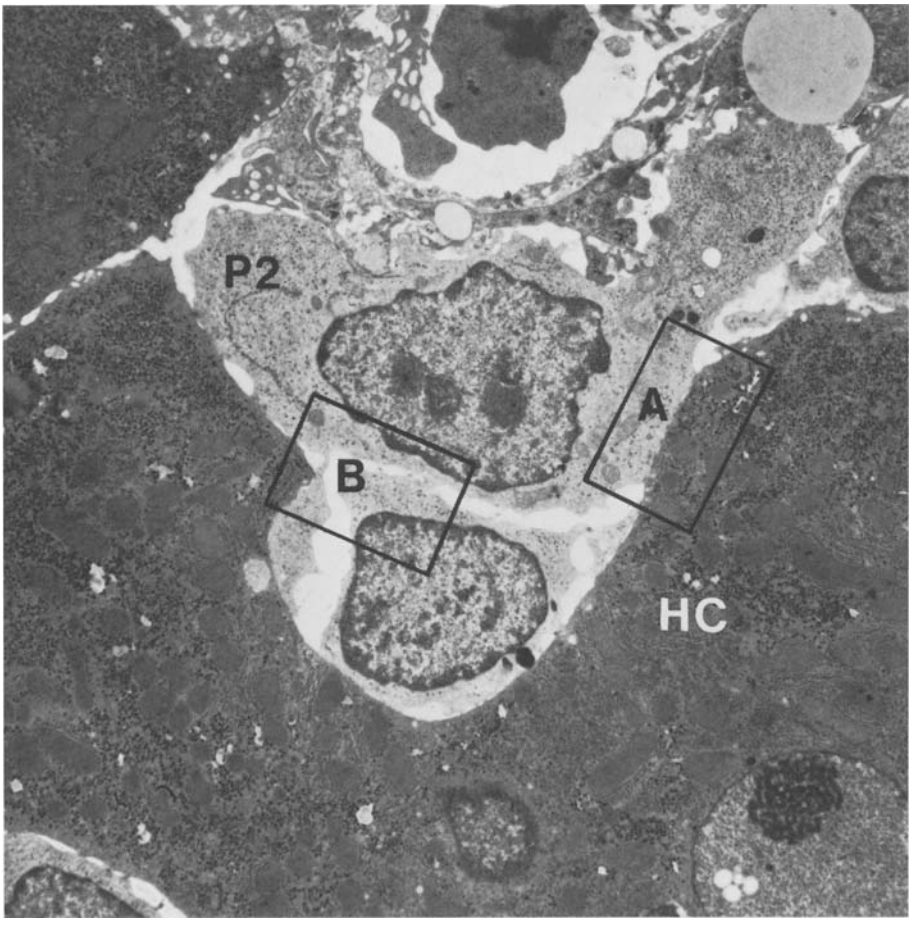
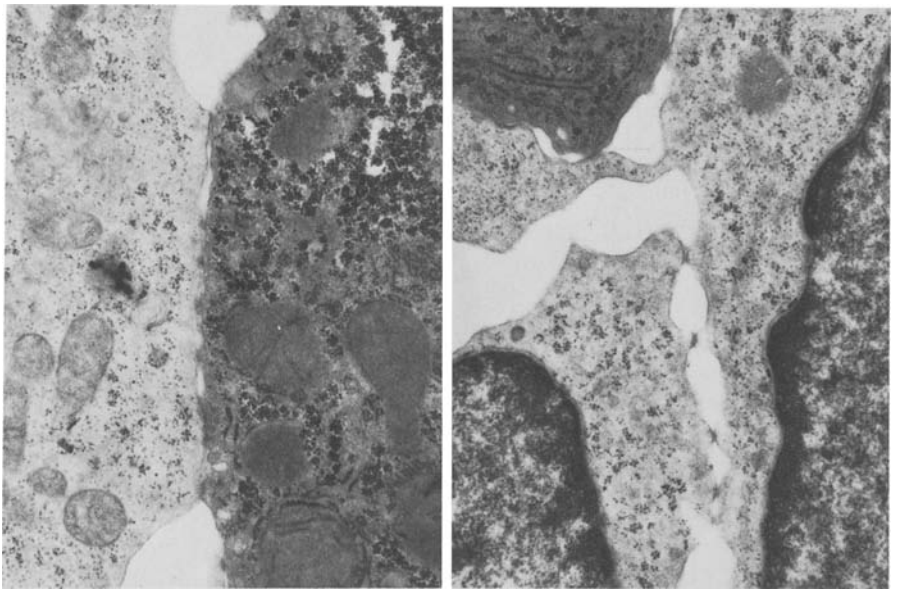


Fig. 4. Type 2 plasmablast (*P2*) in contact with hepatocytes (*HC*). Note irregular shape of cell and nucleus, and prominent nucleoli. For magnified areas in rectangles *A* and *B*, see Figs. 5 and 6. × 3750

were seen in the abundant cytoplasm. A few small lysosomes were occasionally present. Type 2 plasmablasts were usually seen in close contact with macrophages, hepatocytes (Fig. 5), lymphocytes, or other blast cells (Fig. 6). Intercellular contact was characterised by many narrow desmosome-like junctions. Between these junctions there were rounded or ovoid intercellular spaces containing apparently extracellular sacs filled with amorphous material or myelin figures (Fig. 7). Presumed maturation of the blast cells was characterised by 1) a decrease in the size of nuclei which became oval and showed chromatin aggregation as in typical plasma cells, and occasional small nucleoli; 2) a decrease in the number of intercellular junctions, and 3) increase in the number of polyribosomes, with simultaneous formation of rough endoplasmic reticulum throughout the cytoplasm. In this way,



5 6

Fig. 5. Area in rectangle *A* of Fig. 4 magnified to show desmosome-like junctions between blast cell and hepatocyte. ×19,800

Fig. 6. Rectangle *B* of Fig. 4 magnified to show desmosome-like junctions between two blast cells. ×19,800

type 2 plasmablasts appeared to mature into plasma cells of reticular type. Type 2 plasmablasts predominated in fully-developed hepatitis with moderate to severe inflammation.

Type 3. Plasmablasts were 9–12 μm in diameter, oval or slightly elongated, with relatively large nuclei of irregular shape (Fig. 8). These had irregularly dispersed chromatin, nucleoli were inconspicuous, and blebs or indentations were seen at the nuclear membrane. Within the cytoplasm there were numerous monoribosomes, scanty polyribosomes, mitochondria and small Golgi apparatus. Type 3 blasts were exclusively seen in close contact with macrophages, often within sinusoids. Desmosome-like junctions as seen in type 2 blasts were scanty. Maturation appeared to take place during contact with macrophages, and was characterised by 1) a change in the shape of the cells, which became ovoid as well as smaller; 2) nuclei of more regular shape; 3) the formation of rough endoplasmic reticulum, and 4) a marked increase in the number of free polyribosomes, seen in abundance throughout the cytoplasm. In this way there appeared to be a gradual transition to mature plasma cells of the plasmacytoid (lymphatic) type. Neither type 3 blasts nor plasmacytoid plasma cells appeared to transform into plasma cells of the reticular type. Type 3 plasmablasts and lymphatic plasma cells were seen exclusively in type A and B hepatitis with bridging or panacinar necrosis.

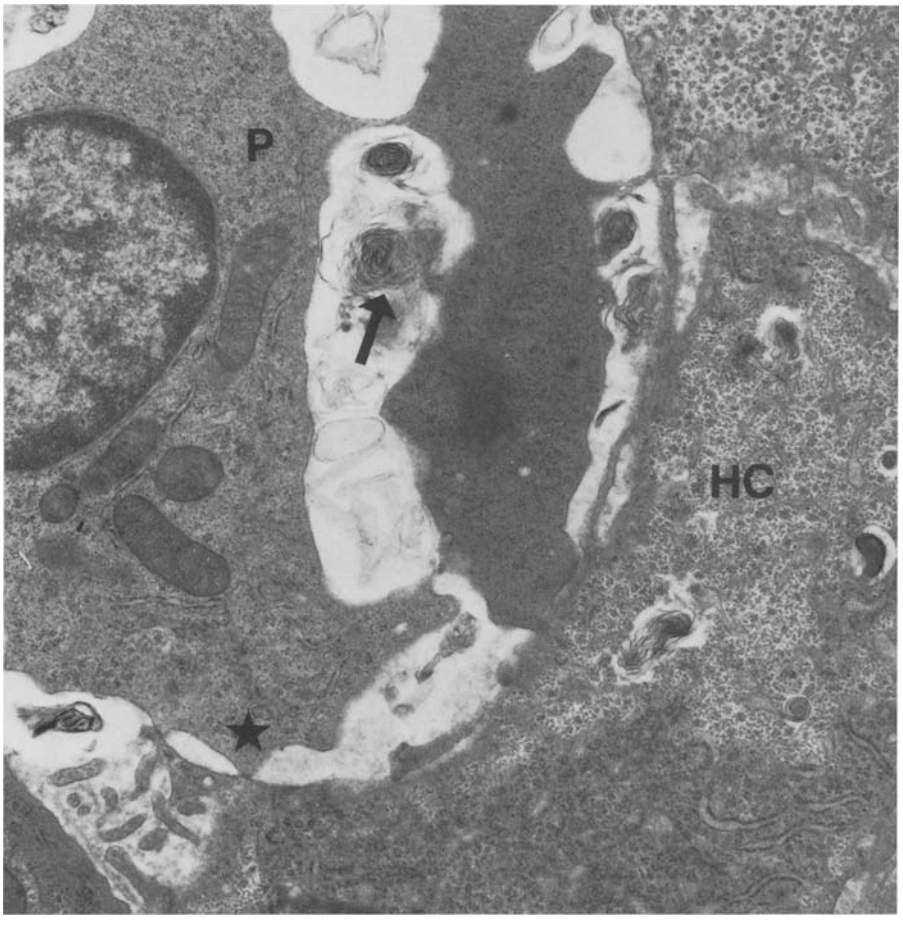


Fig. 7. Scalloped spaces between a hepatocyte (*HC*) and plasmablast (*P*) contain sacs and lamellar structures (*arrow*). Note desmosome-like junctions between the two cells (✦). ×13,500

Type 4. In addition to the three types of blasts described above, there was a clearly different population of cells with plasmablast features. These cells were 7–14 μm in diameter, and had a high nuclear-cytoplasmic ratio, slightly irregular nuclei with dispersed chromatin, and an inconspicuous, centrally located nucleolus (Fig. 9). Some cells were hand mirror- or racket-shaped. Within the cytoplasm, Golgi apparatus was usually located near a nuclear indentation. The most characteristic feature was the presence of long strands of rough endoplasmic reticulum surrounding the nucleus in a regular fashion, with focal whorls where the cytoplasm was wider or where the nucleus was indented. A few lysosomes were occasionally seen. Type 4 blasts were seen in many of the biopsies, but most often in the presence of severe necrosis, usually within necrotic areas. Here they sometimes formed small groups. Although they contained considerable amounts of rough endoplas-

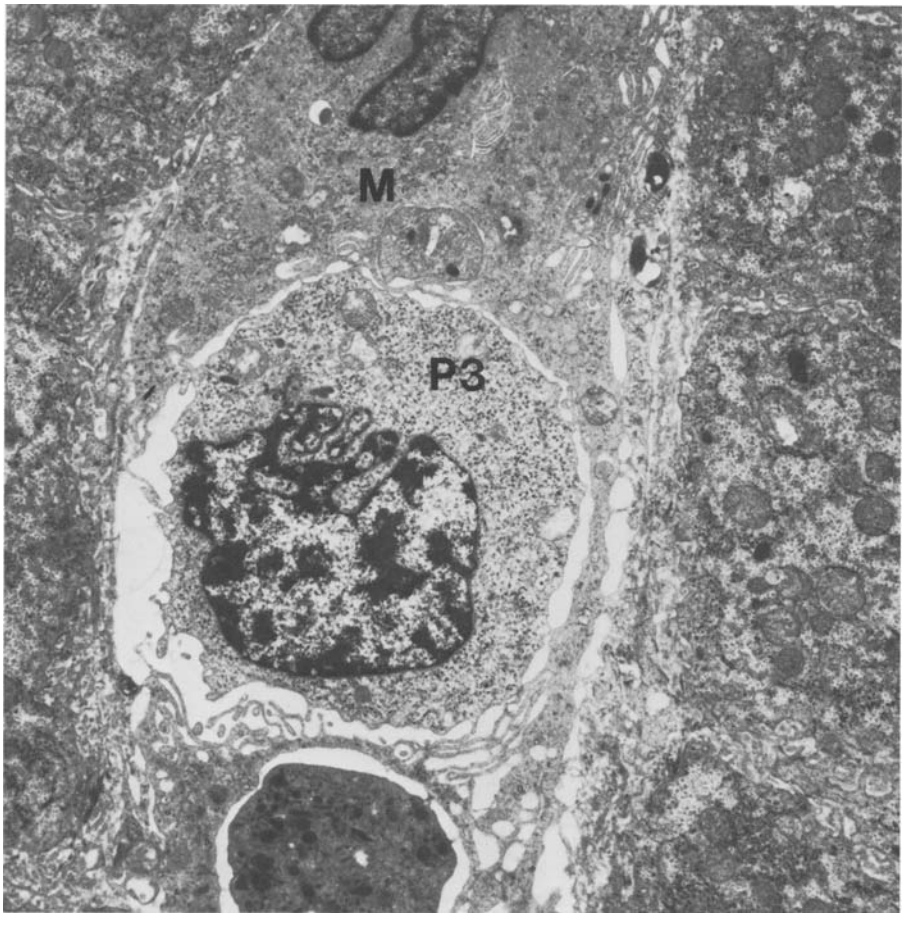


Fig. 8. Type 3 plasmablast (*P3*) within sinusoidal lumen, in close proximity to a macrophage (*M*). The blast has an irregular nucleus with many protrusions and dispersed chromatin. The cytoplasm is rich in polyribosomes. × 5600

mic reticulum, transformation into mature plasma cells of reticular type was not observed.

A few plasmacytoid cells could not be confidently allotted to one of the above categories, because of tangential sectioning or small numbers.

Discussion

Our observations confirm the previous light microscopic finding of plasma cells in the inflammatory infiltrate in a high proportion of cases of acute hepatitis (Mietkiewski and Scheuer 1985). The presence of degenerating or fragmented plasma cells throughout the parenchyma supports the concept that these cells represent an end stage in maturation, and that they die

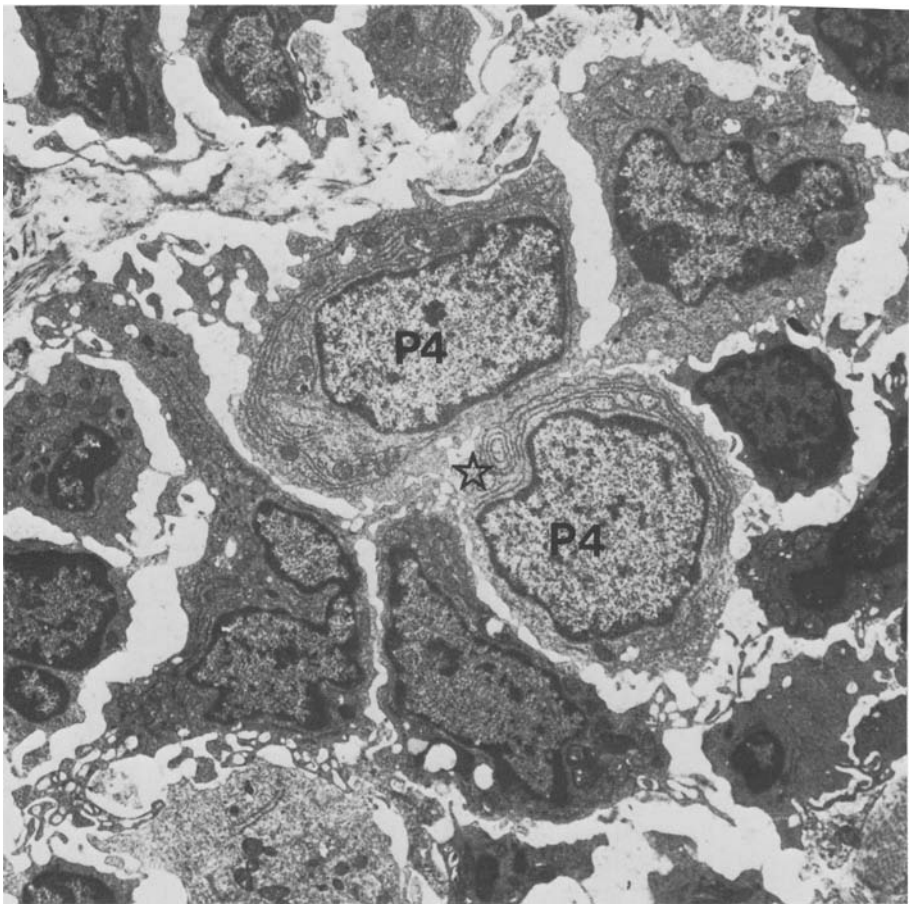


Fig. 9. Two type 4 plasmablasts (*P4*) within an inflammatory infiltrate. Note long strands of rough endoplasmic reticulum parallel to nuclear margin, and whorled endoplasmic reticulum in a nuclear indentation (☆). × 4500

following synthesis and secretion of immunoglobulin. Ultrastructurally the two types of mature plasma cells, reticular and lymphatic, were essentially similar, differing mainly in size. Their mode of formation, however, appeared to be different in so far as this could be deduced from static electron micrographs. Their three precursors, type 1, 2 and 3 plasmablasts, correspond ultrastructurally to lymphoblasts, centroblasts and centrocytes respectively, as described in lymph nodes and in non-Hodgkins lymphoma (Rilke et al. 1978; Lennert 1978). Centroblasts and centrocytes are characteristic of germinal centres after antigenic stimulation, and according to current concepts are able to mature into plasma cells or memory L_2 lymphocytes. It is therefore somewhat surprising that in the present study these cells were seen within mixed inflammatory infiltrates rather than in lymphoid

follicles. However, the finding might explain the occurrence of plasma cells extranodally within inflammatory infiltrates throughout the body.

Type 1 plasmablasts predominated in early acute hepatitis irrespective of aetiology, and maturation appeared to take place within inflammatory infiltrates without significant interaction with other cell types. By contrast, types 2 and 3 were seen mainly in fully developed hepatitis of viral aetiology, usually in the presence of severe necrosis, and maturation seemed to involve contact with other cell types. Plasma cells therefore appeared to be formed in two different ways: by direct transformation of lymphocytes or from centrocytes and centroblasts. Furthermore, type 1 and 2 plasmablasts appeared to transform into reticular plasma cells whereas type 3 formed lymphatic plasma cells. Transition between the two types of mature plasma cells was not observed.

These observations are consistent with the following hypothetical picture of the humoral response in acute hepatitis. The primary immune response, during the first weeks of the disease, involves transformation of type 1 plasmablasts into mature reticular plasma cells within the inflammatory infiltrate. This is in keeping with the observed rise in serum gamma globulin levels in this period (Kaymakcalan et al. 1978; Zhuang et al. 1982). The blast cells may correspond to a subpopulation of lymphoid cells in peripheral blood which transform directly on arrival in the liver. In the case of enteric infection and drug-induced hepatitis, we postulate that the primary immune response takes place in the intestinal immune system, and leads to the formation of plasma cells and memory B_2 lymphocytes. The latter then migrate to the liver and transform directly into plasma cells of reticular type. Type 2 plasmablasts and their derived plasma cells might represent a secondary immune response within the liver involving different antibodies, possibly against self components or host antigens modified by virus. The finding of type 3 plasmablasts in type A and B viral hepatitis suggests that they might participate in immune reactions against viruses. This is in keeping with the observation of Müller-Hermelink and Lennert (1978) that lymphatic plasma cells often appear in large numbers in virus infections such as rubella and in presumed virus infections such as non-specific mesenteric lymphadenitis. They also observed that IgM was demonstrable on the surface of lymphatic plasma cells and that the majority of IgM-producing lymphomas are of lymphatic type; by contrast, IgG- and IgA-producing lymphomas are usually neoplasms of reticular plasma cells. The suggestion that lymphatic plasma cells synthesize IgM antibodies is in keeping with our finding of these cells in type A and B hepatitis, in which serum IgM levels are moderately elevated (Kaymakcalan et al. 1978; Zhuang et al. 1982). Close contact between the blasts and macrophages suggests that the latter may be involved in the stimulation of specific antibody formation as postulated by Wing and Remington (1978), who also stressed the role played by macrophages in bringing about transformation of blast cells.

The abundance of desmosomal junctions between blast cells and hepatocytes or macrophages, associated with sac-like structures, indicates the inti-

mate contact between the various types of cells. Lymphoid cells do not form morphologically detectable junctions under normal conditions, but these have been reported in lymphomas (Imai et al. 1980; Perez-Atayde et al. 1982). Junctions have also been described between lymphoid cells in the bursa of Fabricius (Holbrook et al. 1977).

Our observations suggest that the type 4 plasmablasts represent a different cell line. In general these cells resembled the plasmacytoid T cells (T-associated plasma cells) described in T zones of lymph nodes and in non-Hodgkin's lymphomas (Müller-Hermelink et al. 1973; Müller-Hermelink et al. 1983; Papadimitriou et al. 1983). The cells in our series differed slightly from the latter in their less electron-lucent cytoplasm and lesser tendency to form aggregates. These differences might reflect organ specificity or the different environment. The nature of the type 4 blasts requires confirmation, but their T cell origin is in keeping with the known presence of T lymphocytes in the inflammatory infiltrate of acute hepatitis (Govindarajan et al. 1983; Pape et al. 1983; Shibata et al. 1984). The concept that some cells in the hepatitic infiltrate with the morphological characteristics of plasma cells are of T cell origin is further supported by Mietkiewski and Scheuer (1985), who found plasma cells devoid of immunoglobulins in acute hepatitis both of viral and drug aetiology.

Acknowledgements. The authors are grateful to Miss J. Lewin and Mrs. L. Boxer for expert technical help with electron microscopy and with preparation of figures. We thank our clinical colleagues for access to clinical data. K.A.B. was supported by a grant from the Medical Research Council of the United Kingdom.

References

Bardadin KA, Scheuer PJ (1974) Endothelial changes in acute hepatitis. A light and electron microscopic study. J Pathol 144:213–220

Bernuau D, Rogier E, Feldmann G (1982) A quantitative ultrastructural analysis of the leucocytes in contact with hepatocytes in chronic active hepatitis, with a cytochemical detection of mononuclear phagocytes. Am J Pathol 109:310–320

Cuccurullo L, delVecchio-Blanco C, Manocchio G, Coltorti M (1976) Considerations sur les aspects ultrastructuraux de l'hepatite chronique aggresive. Ann Gastroenterol Hepatol 12:91–105

Govindarajan S, Uchida T, Peters RL (1983) Identification of T lymphocytes and subsets in liver biopsy cores of acute viral hepatitis. Liver 3:13–18

Hadziyannis S, Feizi T, Scheuer PJ, Sherlock S (1969) Immunoglobulin-containing cells in the liver. Clin Exp Immunol 5:499–514

Holbrook KA, Perkins WD, Glick B (1977) Contact sites between lymphoid cells of the bursa of Fabricius: in vivo and in vitro. Anat Rec 189:567–576

Imai Y, Kasajima T, Terashima K, Dobashi M, Matsuda M (1980) Non Hodgkins's malignant lymphoma with special reference to desmosome-like junctions. Acta Pathol Jpn 30:195–217

International Group (1977) Acute and chronic hepatitis revisited. Lancet ii:914–919

Kawanishi H (1977) Morphologic association of lymphocytes with hepatocytes in chronic liver disease. Arch Pathol Lab Med 101:286–290

Kaymakcalan H, Dourdourekas D, Szanto PB, Ainis H, Steigmann F (1978) Immunoglobulins in submassive hepatic necrosis. Am J Gastroenterol 69:289–294

Lennert K (1978) Malignant lymphomas other than Hodgkin's disease. Springer, Berlin Heidelberg New York

Lennert K, Collins RD, Lukes RJ (1983) Concordance of the Kiel and Lukes-Collins classifications of non-Hodgkin's lymphomas. Histopathology 7:549–559

Lukes RJ, Collins RD (1975) New approaches to the classification of the lymphomata. Br J Cancer 31 suppl 2:1–28

Mietkiewski J, Scheuer PJ (1985) Immunoglobulin-containing plasma cells in acute hepatitis. Liver 5:84–88

Müller-Hermelink HK, Lennert K (1978) The cytologic, histologic, and functional bases for a modern classification of lymphomas. In: Lennert K Malignant lymphomas other than Hodgkin's disease. Springer, Berlin Heidelberg New York, p 1–71

Müller-Hermelink HK, Kaiserlink E, Lennert K (1973) Pseudofollikuläre Nester von Plasmazellen (eines besonderen Typs?) in der paracorticalen Pulpa menschlicher Lymphknoten. Virchows Arch [Cell Pathol] 14:47–56

Müller-Hermelink HK, Steinmann G, Stein H, Lennert K (1983) Malignant lymphoma of plasmacytoid T-cells. Morphologic and immunologic studies characterizing a special type of T-cell. Am J Surg Pathol 7:849–862

Papadimitriou CS, Stephanaki-Nikou SN, Malamou-Mitsi VD (1983) Comparative immunostaining of T-associated plasma cells and other lymph node cells in paraffin sections. Virchows Arch [Cell Pathol] 43:31–36

Pape GR, Rieber EP, Eisenburg J, Hoffmann R, Balch CM, Paumgartner G, Riethmüller G (1983) Involvement of the cytotoxic/suppressor T cell subset in liver tissue injury of patients with acute and chronic liver diseases. Gastroenterology 85:657–662

Paronetto F, Rubin E, Popper H (1962) Local formation of gammaglobulin in the diseased liver, and its relation to hepatic necrosis. Lab Invest 11:150–158

Perez-Atayde AR, Chang YC, Seiler MW (1982) Retroperitoneal lymphoma with ribosome-lamellae complexes, cytoplasmic projections and desmosome-like junctions: an ultrastructural study. Ultrastruct Pathol 3:43–49

Rilke F, Pilotti S, Carbone A, Lombardi L (1978) Morphology of lymphatic cells and of their derived tumours. J Clin Pathol 31:1009–1056

Shibata M, Kojima T, Aoyama K, Kouda T, Inoue K, Sasaki H, Imoto S (1984) Morphological studies on the mechanism of hepatocellular injury in type A acute viral hepatitis. Ultrastructural investigation and identification of T cell subsets using peroxidase antibody technique. Acta Hepatol Jpn 25:1–11

Wing EJ, Remington JS (1978) Delayed hypersensitivity and macrophage function. In: Fudenberg HH, Stites DP, Caldwell JL, Wells JV (eds) Basic and clinical immunology, 2nd ed. Lange, Los Altos, pp 195–217

Zhuang H, Kaldor J, Locarnini SA, Gust ID (1982) Serum immunoglobulin levels in acute A, B, and non-A, non-B hepatitis. Gastroenterology 82:549–553

Accepted July 18, 1985