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Interaction of lymphocytes with hepatocytes containing hepatitis B antigen: ultrastructural demonstration of target antigen and T-cell subsets by the peroxidase antibody technique*

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Summary. Hepatitis B virus associated antigens and subsets of lymphocytes in liver tissue were studied using immune electron microscopy to clarify the immune mechanism of hepatocyte lysis in type B chronic hepatitis. Using conventional electron microscopy, infiltrating lymphocytes were observed in direct contact with hepatocytes in areas of piecemeal necrosis and focal necrosis; they showed various types of surface adherence with a contact gap of approximately 20 nm in width. The majority of the hepatocytes that were in contact with lymphocytes could be shown to contain HBsAg and/or HBcAg by immune electron microscopy: HBsAg was localized in the endoplasmic reticulum membranes, in tubular structures, and on the outer coat of Dane particles; HBcAg was observed in the nuclei and in the cytoplasmic matrix of hepatocytes. In some cases HBsAg was observed on the plasma membrane of hepatocyte in contact with lymphocytes. Immune electron microscopy using monoclonal antibodies to subsets of human T-lymphocytes revealed that the lymphocytes in areas of piecemeal necrosis and focal necrosis were predominantly CD 5 or CD 8 positive. In contrast, CD 4 positive cells were infrequently observed in necro-inflammatory regions and Leu 7 positive cells were randomly scattered in the sinusoids away from areas of hepatocyte necrosis. These data suggest that HBsAg is at least one of the target antigens expressed on the hepatocyte membrane possibly enabling cytolytic interaction by cytotoxic T cells in chronic type B hepatitis.

Key words: Immune electron microscopy – T-cell subsets – HBV associated antigens – Lymphocyte interaction

Introduction

The exact mechanism of hepatocyte lysis occurring in patients with HBsAg positive liver disease has not been clarified. The finding of large amounts of hepatitis B virus (HBV) associated antigens in hepatocytes with minimal histological changes in healthy HBsAg carriers suggests that HBV itself has no intrinsic cytopathic effect (Hadziyannis et al. 1973). Immune reactions, especially cell mediated immunity, have been considered to play an important role in the pathogenesis of type B hepatitis (Elsheikh et al. 1978; Warnatz et al. 1979; Hütteroth et al. 1981).

Morphological investigations of immunologically mediated cytolysis demonstrated close contact between cytotoxic lymphocytes and various target cells (Matter 1979; Koren et al. 1973; Liepins et al. 1977; Sanderson and Glauert 1977). The close association of lymphocytes with hepatocytes containing HBV-associated particulate structures suggests that a similar mechanism may be responsible for hepatocyte necrosis in type B hepatitis (Sasaki et al. 1981). For cytolysis to occur, the target antigen must be expressed on the plasma membrane of the target cell and the cytotoxic lymphocytes in contact with the target cells should be immunologically activated to act as killer cells (Burns and Allison 1977).

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The purpose of the present study was to investigate the ultrastructural features of the interaction between lymphocytes and hepatocytes expressing HBV-associated antigens. Immunoperoxidase staining was used to demonstrate viral antigens in hepatocytes and on their plasma membrane and to identify the lymphocyte subsets in areas of liver cell necrosis.

Materials and methods

Conventional and immune electron microscopic investigation was carried out on liver biopsies from 40 patients with persistent HBs antigenaemia. Histological diagnoses were based on an International classification (Review by an International group 1971; 1977). Six were asymptomatic carriers with minimal changes diagnosed as non-specific reactive hepatitis (NSRH), 10 had chronic persistent hepatitis (CPH), 17 had chronic active hepatitis (CAH), and 7 had cirrhosis (LC). HBeAg was present in 22 of the 40 cases and anti-HBe was present in 8.

Anti-HBs goat serum was obtained from the Medical and Biological Laboratories (Nagoya, Japan). Human anti-HBc serum was prepared from a plasma pool of HBsAg carriers. The IgG fractions of these sera were isolated by DEAE-cellulose column chromatography. $F(ab')_2$ fragments were prepared by pepsin digestion, and conjugated with horseradish peroxidase (HRPO) (Sigma HRPO type VI, RZ 3.2) according to the procedure described by Nakane and Kawaoi (1974).

Anti-CD 5 (anti-Leu 1), anti-CD 8 (anti-Leu 2a, anti-OKT-8), anti-CD 4 (anti-Leu 3a) and anti-Leu 7 antibodies (Leu series: Becton Dickinson FACS Systems, Sunnyvale, California, USA, anti-OKT-8: Ortho Pharmaceutical Corp, Raritan, New Jersey, USA) were utilized to detect all T-cells (CD 5), the cytotoxic/suppressor T-cell subset (CD 8), the helper/inducer T-cell subset (CD 4) and the natural killer/killer cell subset (Leu 7) in liver tissue using HRPO-labeled goat antimouse IgG (Dakopatts a/s, Denmark) as secondary antibody in an indirect immunoperoxidase method.

For light and electron microscopic observations small pieces of liver tissue were fixed with a periodate lysine-2% paraformaldehyde (PLP) solution for 4 h, and frozen in tissue Tek OCT compound according to the procedure described by McLean and Nakane (1974); 4 μm thick sections were prepared with a cryostat.

Endogenous peroxidase in the sections was inactivated by 0.005 M periodic acid and 0.1 mg per ml of sodium borohydrate ($NaBH_4$). The sections were then incubated with HRPO-labeled anti-HBs (0.1 mg/ml) and HRPO-labeled-anti-HBc (0.1 mg/ml) for 8 h at 4° C, respectively. For lymphocyte staining, the sections were incubated with anti-CD 5, anti-CD 8, anti-CD 4 and anti-Leu 7 antibodies at a dilution of 1:50, 1:50, 1:50, 1:40, respectively for 12 h at 4° C followed with HRPO-labeled goat anti-mouse immunoglobulins at a dilution of 1:20 for 3 h at room temperature respectively. After washing, slides were stained with diaminobenzidine (DAB)-hydrogen peroxide (H_2O_2) solution.

For immune electron microscopy, sections were stained with DAB-H_2O_2 solution and postfixed in 2.5% glutaraldehyde for 45 min followed by 2% osmic acid for 45 min. They were dehydrated in graded ethanol solutions and embedded in epoxy resin. Specimens for conventional electron microscopy were fixed in 2.5% glutaraldehyde for 1 h followed by 2 percent osmic acid for 2 h, dehydrated, and embedded in an epoxy resin. Ultrathin sections were cut using an LKB-8800 ultramicrotome. Sections, with or without additional staining, were observed by a JEM 200-CX electron microscope.

The histological findings of all biopsy specimens, especially the degree of inflammatory infiltrate, were assessed without knowledge of the clinical diagnosis and laboratory data. Slides immunostained for lymphocyte subsets were prepared in order to estimate the extent of infiltrating lymphocytes both in areas of piecemeal necrosis and of focal necrosis (Table 1); they were arbitrarily graded as follows: −, denotes absence of infiltrating lymphocytes; ±, scarcely observed infiltrating lymphocytes throughout the biopsy specimen; +, randomly observed infiltrating cells; ++, moderate infiltration of lymphocytes as shown in Fig. 5A, B; +++, extensive infiltration of lymphocytes involving nearly all of the periportal and intralobular areas.

In order to assess the percentage of lymphocyte subsets in the necro-inflammatory areas the number of cells of each lymphocyte subset was counted in haematoxylin counterstained serial sections in 32 of the 40 cases; the immunostained cells among 150–200 mononuclear cells were enumerated and expressed as percentages (Fig. 1).

Results

A large number of infiltrating lymphocytes were observed in regions of piecemeal necrosis and focal necrosis. Close contact between lymphocytes and hepatocytes was occasionally encountered at sites where the sinusoidal lining was interrupted and the plasma membranes of the hepatocytes became flat as a result of the disappearance of their villous projections. Nearly one-third of lymphocyte-hepatocyte approximations were of the close contact type. At higher magnification various types of close contact between lymphocyte and hepatocyte were observed: namely, point attachment (Fig. 2A), broad attachment (Fig. 2B, C). At sites of cell contact between both cell types of gap of approximately 20 nm in width was always found (Fig. 2A, C). The thickness of the hepatocyte membrane in the contact area did not differ from that on the remaining surface area and no morphological evidence was found to suggest structural communications between lymphocyte and hepatocyte membranes within the contact area (Fig. 2A, C). The hepatocytes in contact with lymphocytes revealed various degrees of injury: dilatation of the ER, mitochondrial abnormality, and detachment of ribosomes from the ER membranes. Contact of lymphocytes with hepatocytes demonstrating acidophilic degeneration was also found (Fig. 2B, C).

Electron microscopy demonstrated tubular HBsAg structures, 22 nm in diameter, in the dilated cisternae of the ER; core particles, 25 nm in diameter, in the nucleus and/or cytoplasm; and Dane particles, 42 nm in diameter in the dilated cisternae of the ER. These HBV-associated particulate structures were observed in most of the he-

Table 1. T-cell subsets and HBV-associated antigens in liver tissues in chronic B-liver disorders

Patient no.	T-cell subsets in liver tissues								HBV-associated antigens in liver tissues		Histological diagnosis
	Periportal				Intralobular						
	CD5	CD8	CD4	Leu7	CD5	CD8	CD4	Leu7	HBsAg	HBcAg	
1	+	+	+−	−	+	+−	+−	+−	C	−	NSRH
2	+	+	+	−	+	+	+−	+−	C	++	NSRH
3	++	+	+	−	+	+	+−	+−	C	+++	NSRH
4	++	++	+	−	+	+	+	−	C	+++	NSRH
5	++	++	+	−	++	++	+	+−	C	−	NSRH
6	NT	NT	NT	NT	NT	NT	NT	NT	M	NT	NSRH
7	+	+	+−	−	+	+	+−	−	C	+	CPH
8	+	+	+−	−	+	+	+−	+−	C	+++	CPH
9	++	++	+	−	+	+	+−	−	C	−	CPH
10	++	++	+−	−	++	++	+−	+−	C	+++	CPH
11	++	++	+−	−	++	++	+−	+−	C	++	CPH
12	++	++	+−	−	++	++	+−	+−	C	−	CPH
13	NT	NT	NT	NT	NT	NT	NT	NT	M	++	CPH
14	NT	NT	NT	NT	NT	NT	NT	NT	M	++	CPH
15	NT	NT	NT	NT	NT	NT	NT	NT	M	+	CPH
16	++	++	+	+−	++	++	+	+−	C	+++	CAH
17	++	++	+	+−	++	++	+	+−	C	++	CAH
18	++	++	+	+−	++	++	+	+−	C	+	CAH
19	++	++	+	+−	++	++	+−	+−	C	++	CAH
20	++	++	+	+−	++	++	+	+−	C	−	CAH
21	++	++	NT	+−	++	++	NT	+−	C	+	CAH
22	++	++	+	+−	++	++	+	+−	C	++	CAH
23	++	++	+	−	++	++	+−	+−	C	+++	CAH
24	++	++	+−	−	+	+	+−	−	C	++	CAH
25	++	++	+−	−	++	+	+−	+−	C	−	CAH
26	++	++	+−	−	+	+	+−	+−	C	+	CAH
27	++	+	+	−	+	+	+−	−	C	++	CAH
28	+	+	+−	−	+	+	+−	+−	C	−	CAH
29	+++	++	+−	+−	+++	++	+−	+−	C	+++	CAH
30	NT	NT	NT	NT	NT	NT	NT	NT	M	+	CAH
31	NT	NT	NT	NT	NT	NT	NT	NT	M	++	CAH
32	NT	NT	NT	NT	NT	NT	NT	NT	M	+	CAH
33	NT	NT	NT	NT	NT	NT	NT	NT	M	+	CAH
34	++	++	+	+−	++	++	+	+−	M	++	LC(active)
35	++	++	++	+−	++	++	+	+−	C	+	LC(active)
36	++	++	+	+−	++	++	+	+−	C	−	LC(active)
37	++	++	+	−	++	++	+	−	C	−	LC(active)
38	++	++	+	+−	++	++	+	+−	C	−	LC(inactive)
39	+	+	+−	−	+	+	+−	+−	C	+	LC(inactive)
40	+	+	+−	+−	+	+	+−	+−	C	+	LC(inactive)

The semi-quantitative assessment of the number of infiltrating lymphocytes per biopsy is indicated by: − (none); +− (very rare); + (rare); ++ (moderate); +++ (numerous). The abbreviations used are: NSRH: non-specific reactive hepatitis; CPH: chronic persistent hepatitis; CAH: chronic active hepatitis; LC: liver cirrhosis. M: membranous localization of HBsAg; C: cytoplasmic localization of HBsAg; NT: not tested

patocytes with close contact relationship to lymphocytes. Although the frequency was different in each case, more than 50% of the hepatocytes in contact with lymphocytes contained HBV-associated particulate structures: Dane particles in the cisternae of ER and core particles in the nucleus and/or in the cytoplasmic matrix were the most frequent (nearly 50%), whereas tubular structures alone were less frequent (less than 10%).

Immunoperoxidase staining revealed two different patterns of localization of HBsAg in hepatocytes: diffuse membranous localization and intracytoplasmic localization (Fig. 3A, B). The haematoxylin counterstained slides immunostained for HBsAg clearly demonstrated the close association between hepatocytes containing HBsAg and inflammatory cells in 25 of 40 cases (62.5%); cytoplasmic HBsAg was frequently observed (21 cases)

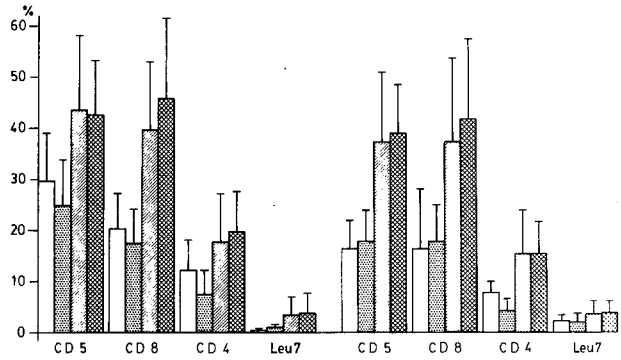

Fig. 1. Percentage of lymphocyte subsets of the inflammatory cells in regions of piecemeal necrosis and focal necrosis in chronic B-liver disorders. *Piecemeal necrosis:* ☐ non-specific reactive hepatitis (NSRH); ▨ chronic active hepatitis (CAH). *Focal necrosis*: ▩ chronic persistent hepatitis (CPH); ▩ liver cirrhosis (LC)

in the regions of piecemeal necrosis and focal necrosis, furthermore, membranous HBsAg was also observed in such areas although less frequently (4 cases). By immune electron microscopy, the membranous localization of HBsAg was found to be associated with small of HBsAg reaction product on the ER membranes and on the outer coat of the Dane particles (Fig. 4A). Intracytoplasmic localization of HBsAg was demonstrated on the membrane of the ER containing either tubular structures or Dane particles as well as on the latter particles themselves (Fig. 4C). Cell membrane localization of HBsAg was encountered in 9 of our 40 cases (Table 1): NSRH (1/6), CPH (3/9), CAH (4/18), and LC (1/7). Close contact between lymphocytes and hepatocytes expressing HBsAg on the plasma membrane was observed in several cases of CPH and CAH (Fig. 4B), but was only rarely observed in NSRH and LC. Close contact between lymphocytes and hepatocytes containing cytoplasmic HBsAg on their ER membrane but not on their plasma membrane was also found (Fig. 4C). HBcAg was localized both in the nuclei and in the cytoplasmic matrix of hepatocytes. Most of the hepatocytes in contact with the lymphocytes revealed HBcAg in their cytoplasmic matrix (Fig. 5).

Most of the lymphocytes in areas of piecemeal necrosis and focal necrosis as well as those in the portal tracts were found to be CD 5, or CD 8 positive cells (Fig. 6A, B) whereas CD 4 positive cells were scarce in these areas. As shown in the Table and in Fig. 1, CD 5, or CD 8 positive cells were observed more frequently in CAH and active cirrhosis (nearly 40%) than in cases of NSRH, CPH and inactive cirrhosis (less than 30%). The percentages of CD 5 and CD 8 positive cells were almost the same in areas of piecemeal necrosis and in focal necrosis. On the contrary, CD 4 positive cells were the minor component (less than 20%) of the cellular infiltrate in these areas, whereas they tended to accumulate in the central part of enlarged portal tracts. Few Leu 7 positive cells were observed, mainly in the sinusoids.

The lymphocytes present in regions of piecemeal necrosis and focal necrosis were predominantly CD 5, or CD 8 positive and demonstrated close contact with hepatocytes (Figs. 7–9). In contrast, contact between CD 8 negative lymphocytes and hepatocytes in the parenchymal lesions was rarely observed in the present study (Fig. 9). Only Leu 7 positive cells were found in the sinusoids and no contact between them and hepatocyte could be demonstrated.

Discussion

In a study of chronic liver disease, Kawanishi (1977) reported various types of morphological association between lymphocytes and hepatocytes, suggesting a cell-mediated immune process. More recently, Bernuau et al. (1982) made a quantitative analysis of leucocytes in contact with hepatocytes in HBsAg-negative chronic active hepatitis and emphasized mononuclear phagocyte mediated mechanisms in hepatocyte necrosis. In our study of type B hepatitis, the infiltrating lymphocytes in contact with hepatocyte membranes demonstrated various types of surface adherence with a contact gap of approximately 20 nm in width (Sasaki et al. 1981). The mechanism of the cell lysis induced by cytotoxic T-lymphocytes (CTL) has been thought to be due to disruption of the plasma membrane of the target cell (Henny 1973). However, a recent electron microscopic examination of CTL-mediated cell death demonstrated intracellular disintegration of the target cell nucleus (Russell et al. 1980; Russell and Dobos 1980). The hepatocytes in contact with the lymphocytes revealed various degrees of injury: dilatation of the ER, mitochondrial abnormality, detachment of the ribosomes from the ER membranes, disappearance of the villous projections of the plasma membrane, and acidophilic degeneration.

For these immunopathological reactions to occur, it is considered necessary that the target antigen should be expressed on the plasma membrane of the target cells. The possibility of HBsAg as a target antigen in type B hepatitis has been supported by studies using immuno-fluorescent methods (Ray et al. 1976) and the lymphocytotoxicity test against either HBsAg coated heterologous tar-

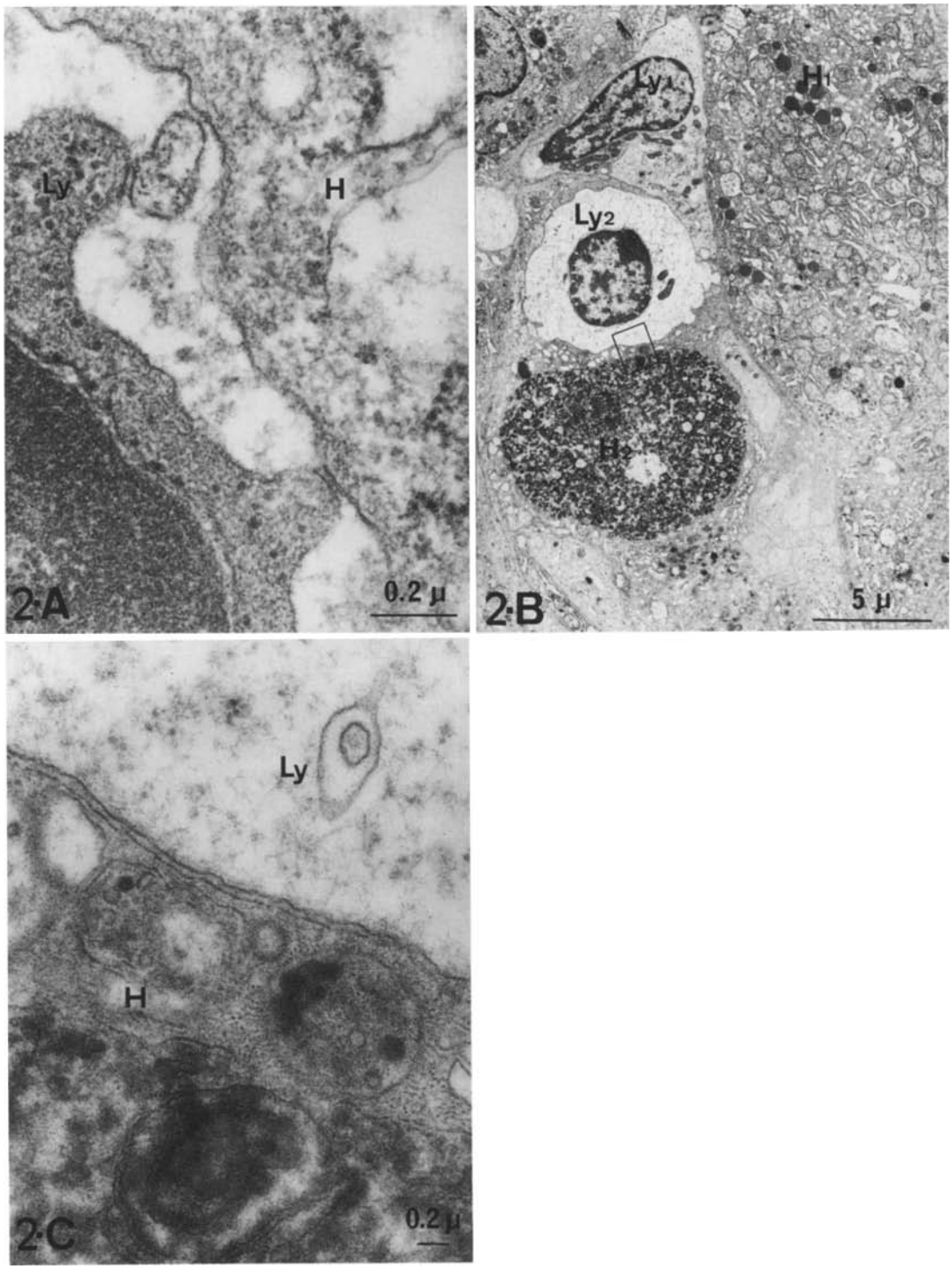

Fig. 2. **A** Point contact of lymphocyte (*Ly*) with hepatocyte (*H*) with a contact gap of approximately 20 nm in width. Uranyl acetate and lead citrate (×66250). **B** Broad contact of lymphocytes (Ly_1, Ly_2) with hepatocyte (H_1) and hepatocyte in acidophilic degeneration (H_2). Uranyl acetate and lead citrate (×3600). **C** Higher magnification of **B.** Broad contact of lymphocyte (*Ly*) with hepatocyte in acidophilic degeneration (*H*) with a contact gap of approximately 20 nm in width. Uranyl acetate and lead citrate (×24000)

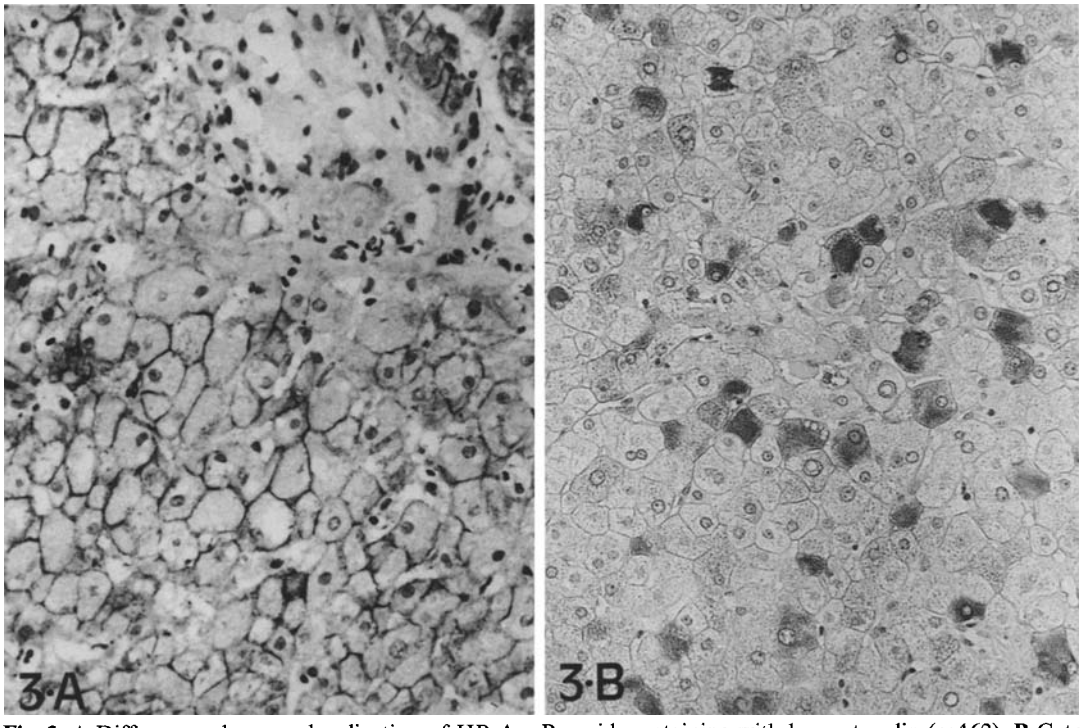

Fig. 3. **A** Diffuse membranous localization of HBsAg. Peroxidase staining with haematoxylin (×162). **B** Cytoplasmic localization of HBsAg. Peroxidase staining with haematoxylin (×162)

get cells (Alberti et al. 1977) or autologous hepatocytes (Eddleston et al. 1982). Mondelli et al. (1982) have suggested that HBcAg is a target antigen for T-cell mediated cytotoxicity in HBsAg positive chronic liver disease because of their finding of a significant inhibition of the cytotoxic reaction in the autologous lymphocytotoxicity assay after adding anti-HBc IgG. Moreover, Trevisan et al. (1982) observed HBcAg on the plasma membrane in isolated hepatocytes in type B hepatitis after antibody elution with high molar urea and suggested that HBcAg is a target of cell-mediated cytotoxicity. Our previous study demonstrated the presence of HBsAg reaction products on the plasma membrane in addition to localization on the tubular structures, outer coat of the Dane particles, and the ER membrane (Kojima 1982). Close contact between lymphocytes and hepatocytes showing HBsAg on their plasma membrane was also observed, thus suggesting that the HBsAg expressed on the plasma membrane may be one of the target antigens (Sasaki et al. 1982).

Concerning the localization of HBcAg, the hepatocytes in contact with lymphocytes contained HBcAg in their cytoplasmic matrix, but not on their plasma membrane. Further investigation is needed to confirm whether HBcAg localized on the plasma membrane is a target antigen, as a membrane bound immunoglobulin-G may be able to hide such membrane-expressed antigens. Recently, one of us (Kojima et al. 1984) demonstrated the HBcAg on the plasma membrane of hepatocytes in a study of Italian patients. On the other hand, HBV-associated neoantigen (Galbraith and Fudenberg 1977), the liver specific protein (LSP) (Meyer zum Büschenfelde et al. 1979), the liver membrane antigen (LMAg) (Hopf et al. 1976) might also be considered as candidate target antigens, especially in case of close contact between lymphocytes and hepatocytes without membranous expression of HBV-associated antigens.

In recent years, newly developed monoclonal antibodies to human T-lymphocytes and subsets have been used to elucidate the immune mechanism in liver diseases. Govindarajan et al. (1983) in acute viral hepatitis and Pape et al. (1983) in acute and chronic liver diseases, have identified the lymphocytes present in cryostat sections of liver biopsies and concluded that the cytotoxic/suppressor T-cell subset is the dominating infiltrating cell and takes part in lymphocyte mediated hepatocyte lysis. In our study with monoclonal antibodies to human T-lymphocytes, most of the infiltrating lymphocytes were noted to be CD 5 or CD 8 positive cells, in areas of piecemeal necrosis and focal necrosis as well as in the portal tracts.

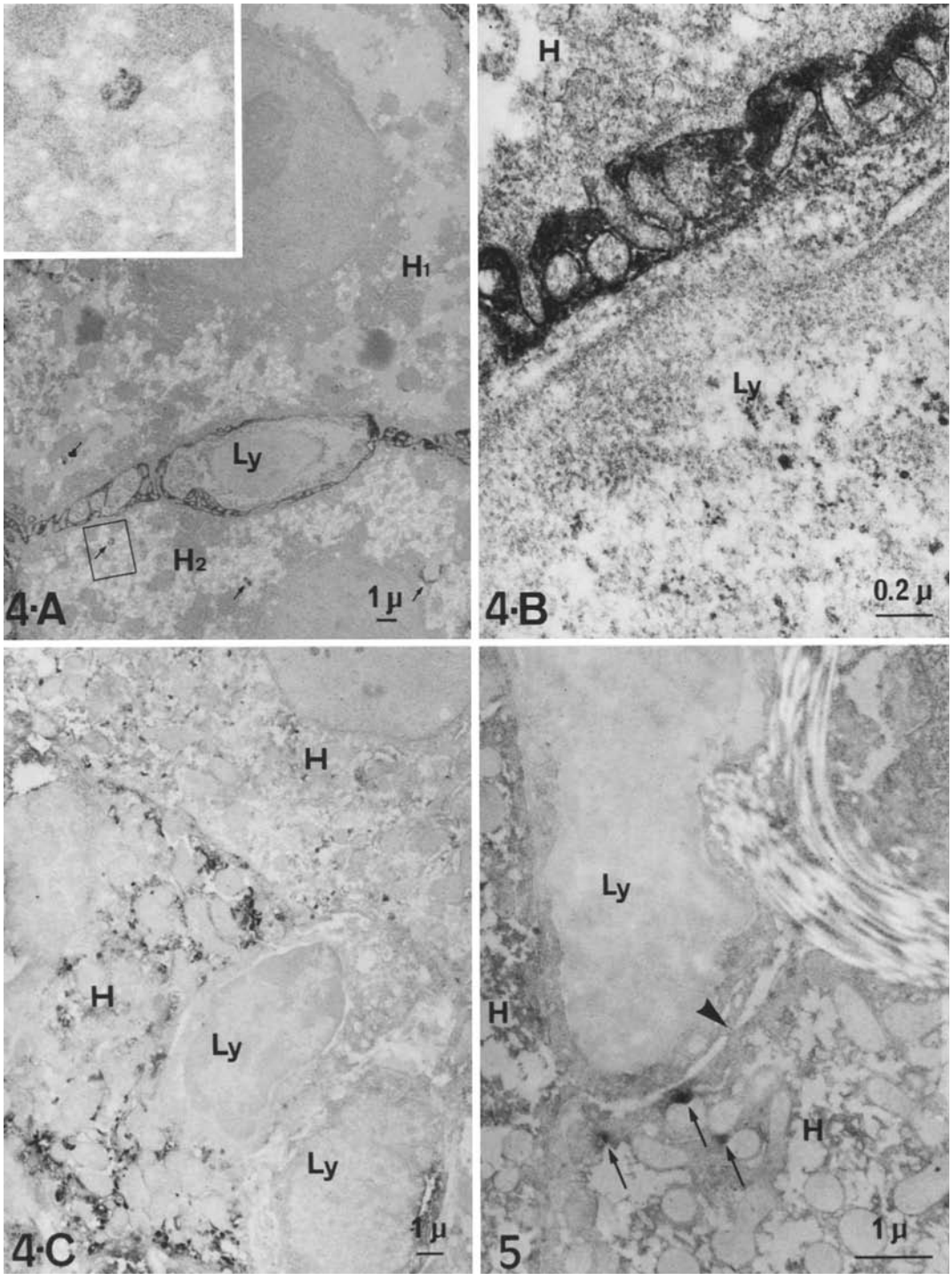

Fig. 4. **A** Close contact between lymphocyte (*Ly*) and hepatocytes (H_1, H_2) showing HBsAg on the plasma membrane in addition to some reaction products on *ER* (*arrows*) of the hepatocytes. Peroxidase staining (×4000). *Insert*: Higher magnification of HBsAg staining in the ER. Peroxidase staining. (×1640). **B** Higher magnification of close contact between lymphocyte (*Ly*) and hepatocyte (*H*) showing HBsAg on the plasma membrane. Peroxidase staining with lead citrate counterstaining (×43200). **C** Contact of lymphocyte (*Ly*) and hepatocyte (*H*) showing HBsAg on some cisternae of the ER. No reaction products of HBsAg on the plasma membrane of the hepatocytes. Peroxidase staining (×4000)

Fig. 5. Point contact (*arrowhead*) between lymphocyte and hepatocyte containing HBcAg reaction products (*arrows*) in the cytoplasmic matrix. Peroxidase staining (×11600)

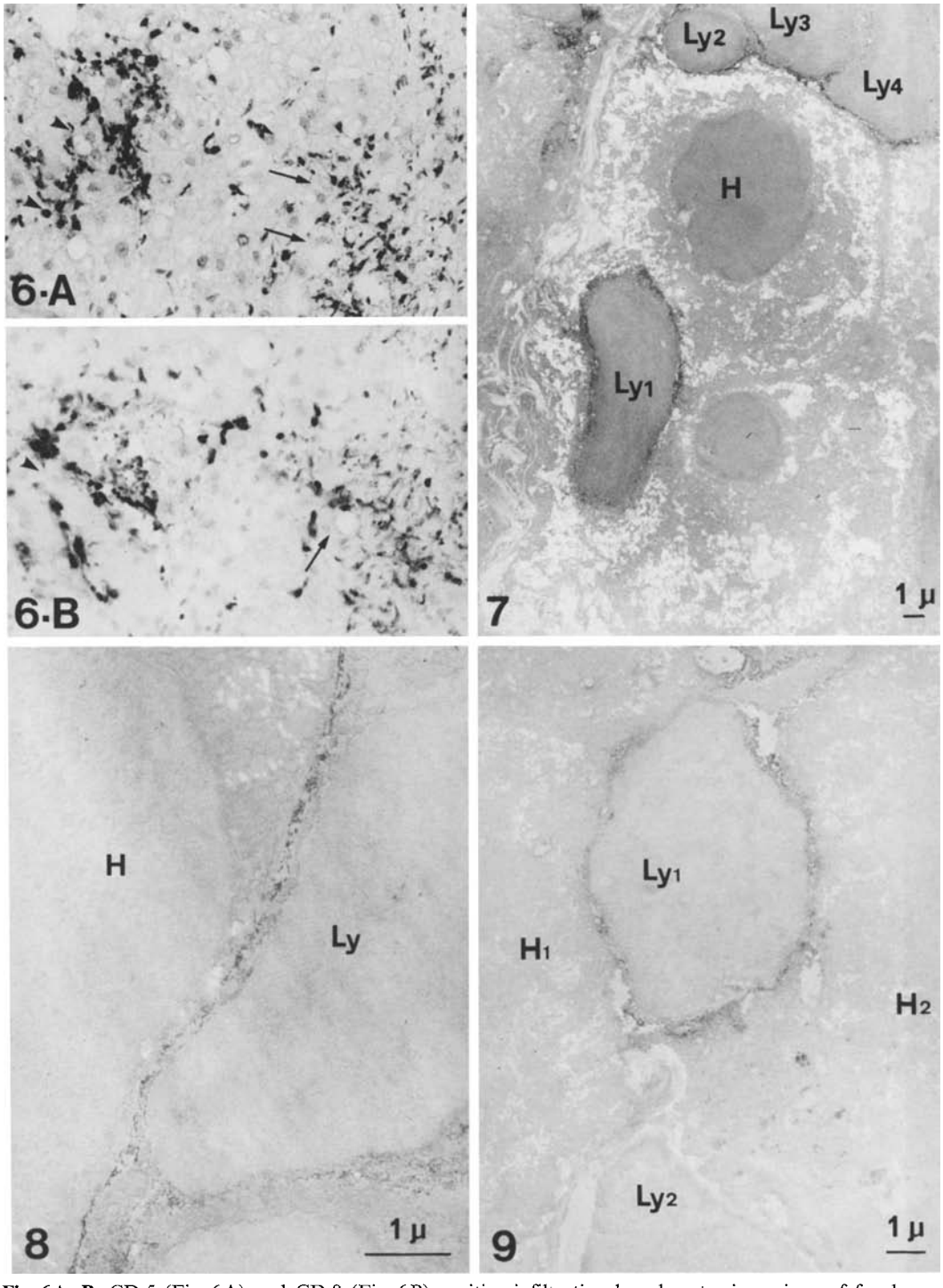

Fig. 6A, B. CD 5 (Fig. 6A) and CD 8 (Fig. 6B) positive infiltrating lymphocytes in regions of focal necrosis (*arrow head*) and piecemeal necrosis (*arrow*) as well as in the portal tracts. Serial sections. Peroxidase staining with haematoxylin counterstaining (× 52)

Fig. 7. Hepatocyte (*H*) surrounded with CD 8 positive lymphocytes (L_1, L_2, L_3, L_4) in area of piecemeal necrosis (× 4000)

Fig. 8. Contact between hepatocyte (*H*) and CD 5 positive lymphocyte (*Ly*). Peroxidase staining. (× 12500)

Fig. 9. CD 8 positive lymphocyte (Ly_1) in contact with hepatocyte (*H*). Note absence of contact between the CD 8 negative lymphocyte (Ly_2) and hepatocytes in a region of focal necrosis. Peroxidase staining (× 5840)

Though anti-CD 8 antibody detects both cytotoxic and suppressor cells, the cells in these regions are considered to be cytotoxic because they are localized in regions with hepatocellular damage. Furthermore, using immune electron microscopy, CD 8 positive cells were observed to be the type of lymphocyte in close contact with those hepatocytes showing degeneration of their organelles in areas of piecemeal necrosis and focal necrosis (Sasaki et al. 1984), resembling the findings in in vitro studies of CTL (Matter 1979; Koren et al. 1973; Liepins et al. 1977; Sanderson and Glauert 1977). However, close contact between CD 8 negative lymphocytes and hepatocytes was observed rarely. This might be due to the fact that these lymphocytes form a minor component of the cellular infiltrate. Moreover, CD 4 positive cells were observed infrequently in the necrotic regions, and did not come in contact with hepatocytes. Similarly Leu 7 positive cells were observed randomly in the sinusoids away from areas of hepatocyte necrosis (Yamada et al. 1984). Montano et al. (1983) suggested immune mechanisms in piecemeal necrosis and in focal necrosis based on the results of their study of the ratio of inducer to cytotoxic/suppressor cell in liver tissues with autoimmune and HBV-induced chronic liver disease. In contrast, Si et al. (1984) examined the composition of the infiltrating lymphocytes present in areas of piecemeal necrosis and concluded that the lymphocytes in piecemeal necrosis were predominantly cytotoxic and that T-cell mediated cytotoxicity was the principal mechanism of immunological damage, regardless of the aetiology. In agreement with this in our study of type B hepatitis, CD 8 positive cells were the predominant cells demonstrated in areas of piecemeal necrosis and focal necrosis, and were found to have contact with hepatocytes. Moreover, since a close association between HBsAg positive hepatocytes and inflammatory cells at the light microscopic level was found in necro-inflammatory regions, close contact of CD 8 positive lymphocytes appears to be more frequent with HBsAg positive hepatocytes than with HBsAg negative ones.

As mentioned above, conventional electron microscopic examinations have demonstrated direct contact of lymphocytes with target cells during in vitro lymphocyte mediated cytolysis (Biberfeld and Johansson 1975). In addition, the close contact of lymphocytes with hepatocytes has been considered to be the morphological characteristic of the immune reaction in the development of type B hepatitis (Sasaki et al. 1981). Because immune electron microscopy using monoclonal antibodies reveals that most of the infiltrating lymphocytes in areas of piecemeal necrosis and focal necrosis are CD 5 and CD 8 positive and close contact of these lymphocytes with hepatocytes was often encountered, cytotoxic T-cells are probably important in the process of hepatocyte lysis during the course of type B hepatitis.

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