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Cytoplasmic Fusions between Liver Parenchymal Cells and Infiltrating Cells in Chronic Aggressive Hepatitis*

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Summary. Lymphocytes and plasma cells have been demonstrated in the peripheral zones of lobules in liver biopsies from patients suffering from chronic aggressive hepatitis. In the region of contact between lymphocytes and parenchymal cells, the cell membranes are frequently not demonstrable so that an impression is created of cytoplasmic fusion. However, when the goniometer is used, the cell membranes in this region can still be demonstrated at a certain tilt angle. It can therefore be concluded that the presumed cytoplasmic fusions are frequently the result of superposition effects in projection.

Organ specific antibodies and cellular immune mechanisms appear to be important in the pathogenesis of chronic aggressive hepatitis (Tobias *et al.*, 1967; Gelzayd *et al.*, 1967; Warnatz *et al.*, 1969; Popper *et al.*, 1970; Doniach, 1972). In electron microscopic studies of this condition, plasma cells and lymphocytes in various stages of activity can be observed in Disses' spaces, often in close contact with the parenchymal cells (Schaffner *et al.*, 1963; Paronetto *et al.*, 1969; Pisi *et al.*, 1970; Orcel *et al.*, 1972). A similar behaviour of the lymphatic cells characterizes the morphological picture of the acute rejection of allogenic organ transplants and autoimmune diseases (Klion *et al.*, 1967; Brandes *et al.*, 1969; Roessner *et al.*, 1971).

In areas of such lymphoepithelial junctions, the cell membranes of the infiltrating and parenchymal cells are frequently not visible. These cytoplasmic fusions are repeatedly believed to be the morphological expression of immunologically induced cell damage (Wiener, 1970).

This paper deals with cell junctions between parenchymal and infiltrating cells in chronic aggressive hepatitis. Using a goniometer stage cell junctions were examined and the observations will be discussed.

Material and Methods

15 liver biopsies of patients with clinically and histologically confirmed chronic aggressive hepatitis were prepared for investigation by electron microscopy. The specimens were fixed in 2.25% glutaraldehyde (0.05 m phosphate buffer pH 7.4) for a period of 2 hours at 4° C and then washed in the buffer for 24 hours. Post fixation was carried out in 1.33% osmic acid in 0.05 m phosphate buffer pH 7.4 for 2 hours. The tissue was dehydrated in an alcohol series and embedded in Epon 812. Sections were cut with a Porter Blum ultra microtome, mounted on 3 mm copper grids and post stained for 15 min with 5% uranyl acetate and for 3 min with

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lead citrate. The specimens were studied with the Philips EM 300 at 80 kV using the double condenser.

So as to eliminate projection superposition effects, the EM 300 was equipped with a goniometer stage. Semi thin sections stained with Toluidin blue were used for light microscope comparisons.

Results

In the biopsies which we studied, lymphocytes and, exceptionally, some plasma cells were present in Disses' spaces particularly in the peripheral zones of the lobules. The average diameter of the majority of the lymphocytes observed was 5 μm. These small lymphocytes are characterized by an oval nucleus, rich in chromatin, with a narrow cytoplasmic zone and few cell organelles. The number of lymphocytes with an average diameter of 8 μm is much smaller. The nucleus of these cells has an irregular configuration and frequently contains 2–3 nucleoli. Ribosomes and cell organelles, such as mitochondria and endoplasmic reticulum are more numerous in these cells, compared with the small lymphocytes. As a result of the narrow space between infiltrating cells and the liver parenchymal cells, the latter are deformed. Thus, in areas of cellular contacts, the microvilli of the liver parenchymal cells are not developed (Fig. 1 a).

Over large areas, the cell membranes cannot be clearly imaged. The membranes of the parenchymal cells and lymphocytes can frequently not be distinguished from one another so that an impression is created of cytoplasmic fusion (Fig. 1 a). In this area, when using a Goniometer stage which permits optimum orientation of the specimen with respect to the electron beam, normal cell membrane structures can be demonstrated (Fig. 1 b).

Discussion

The results have indicated that in Disses' spaces of all biopsies studied plasma cells and lymphocytes can be demonstrated (Schaffner *et al.*, 1963; Pisi *et al.*, 1970). The lymphocytic infiltration suggests that cellular immune mechanisms are involved in the pathogenesis of the chronic aggressive hepatitis (Paronetto *et al.*, 1969). A positive result of the lymphocyte transformation test and of the leucocyte migration inhibition test supports this opinion (Soborg *et al.*, 1967; Tobias *et al.*, 1967; Warnatz, 1969; Miller *et al.*, 1972). The observation that children with a-gamma-globulinemia also suffer from chronic aggressive hepatitis underlines the importance of the cellular immune mechanism in this condition (Good *et al.*, 1960).

Morphological investigations of allogenic organ transplants emphasize the relationship between lymphocytic infiltrates and the parenchyma of the grafted organ (Kountz *et al.*, 1963; Porter *et al.*, 1964; Wiener *et al.*, 1964; Klion *et al.*, 1967; Rosenau *et al.*, 1969; Wiener, 1970). Comparable observations were made on thyroid gland tissues of patients with Hashimotos' thyreoiditis (Irvine *et al.*, 1963; Brandes *et al.*, 1969; Neve, 1969).

In the area of the contact zone between parenchymal cells and lymphocytes fusions of the cell membrane were often described (Kountz *et al.*, 1963; Porter

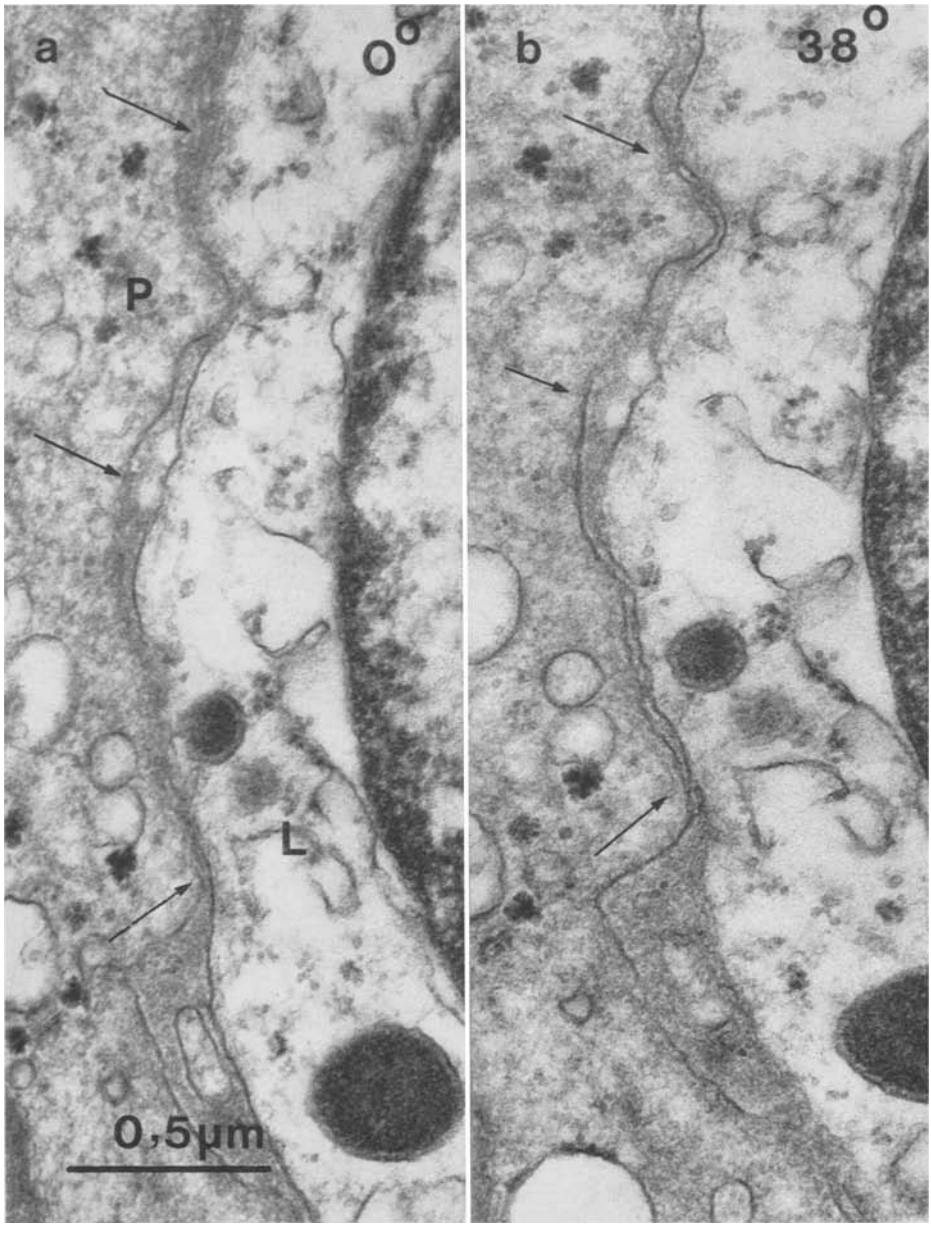

Fig. 1. a Lymphocyte (*L*). Liver parenchymal cell (*P*). Membrane defects (arrows). b After tilting through 38° one can observe membranes in the area of cytoplasmic fusion (arrows). Magn.: ×55000

et al., 1964; Wiener *et al.*, 1964; Brandes *et al.*, 1969). The cell membranes of the enterocytes and of the intraepithelial lymphocytes in idiopathic steatorrhoea have a similar appearance (Otto *et al.*, 1971, 1972).

Such modified cell membranes are interpreted as being morphological manifestations of an antigen-antibody reaction (Wiener, 1970). In this connection, it should be mentioned that these types of fusion could not be demonstrated by other authors (Klion *et al.*, 1967; Blackburne *et al.*, 1968; Neve, 1969; Rosenau *et al.*, 1969).

When using the goniometer which permits optimum orientation of the specimen with respect to the electron beam, cell membranes were demonstrated in the region of membrane defects studied by us after setting the correct tilt angle. We believe that the phenomena considered to be cell fusions might be caused by projection superposition effects in the microscope.

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