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Pancreatic duct infiltration in the low-dose streptozocin-treated mouse

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Summary. The pancreatic-duct system was observed during the initial stage of type 1 diabetes in C57BL/6J mice rendered diabetic with low doses of streptozocin. Light microscopy revealed that the ducts located in close proximity to islets (islet ducts) were involved in the infiltrating process: inflammatory cells extended from the islets to these ducts. However, ducts that were located far from islets (non-islet ducts) were generally free from infiltration. Immunocytochemistry revealed that both islet ducts and non-islet ducts express MHC class II and ICAM-1 molecules: this positivity seems to be mainly expressed by elements infiltrating the connective layer or by endothelia of vessels surrounding ducts. Strong ICAM-1 positivity demonstrates that adhesiveness is widely represented in early diabetes in this animal model. At the ultrastructural level only a few endocrine elements were observed scattered within the epithelial layer and single infiltrating elements were rarely encountered within the connective layer of ducts. The existence of other sites of «activation» other than the islets of Langerhans, in this as well as in other animal models of types 1 diabetes, is consistent with the hypothesis of an initially more widespread and less specific process that later undergoes restriction.

Key words: Exocrine ductules, Infiltration, Streptozocin, Type 1 diabetes

Introduction

Infiltration of the pancreatic ductules in the exocrine portion of the gland has been reported to occur in the non obese diabetic (NOD) mouse: this ductulitis is concurrent to a perivasculitis and both these phenomena are seen together with infiltration of the islets of Langerhans (Papaccio et al., 1993a).

This also seems to occur in the low-dose streptozotocin-treated (LDS) mouse, albeit with less homogeneity and intensity, but nobody has, up to now, concentrated their attention on this event, probably due to the thought that it would be of minor interest.

In this animal model of type 1 diabetes an increased expression of class II immunoreactive cells within infiltrated islets has been reported (Papaccio et al., 1991). Moreover, during the initial stages of infiltration, margination of blood monocytes and subsequent diapedesis with transformation into activated tissue macrophages has also been described (Papaccio and Esposito, 1992). Intercellular adhesion molecule-1 (ICAM-1) is a ligand that serves to bind lymphocytes and possibly monocytes and polymorphonuclear leukocytes to endothelia (Marlin and Springer, 1987).

Therefore, we studied the ductular infiltrate and the expression of MHC class II and ICAM-1 molecules at this level in the early stages of LDS-induced type 1 diabetes.

Materials and methods

25 male C57BL/6J mice, aged 8-10 weeks, weighing 28-35 g, fed ad libitum, were used for the experiment. 20 animals were treated with 40 mg/Kg b.wt. Streptozocin (Upjohn Co., Kalamazoo, MI, USA) given intra-peritoneally on 5 consecutive days (LDS-treatment), using the Like and Rossini schedule (1976). The remaining 5 were used as controls.

Animals were checked for blood glucose on the day before the start of the LDS treatment (day 0), then before killing (day 15): blood was obtained from the retro-orbital plexus and measurements taken using the hexokinase method (Boehringer, Mannheim, Germany). Mice were decapitated under ether anaesthesia and their pancreases excised and processed for morphological studies. For light microscopy samples of the pancreases were fixed in Bouin's fixative and embedded in paraffin. Serially sectioned slides (5 µm thick) were stained with haematoxylin-eosin.

For immunocytochemistry, cryocut sections were stained by the avidin-biotin peroxidase indirect staining method as previously described (Papaccio et al., 1991).

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Slides were incubated for 12 hours at 4 °C with primary antibodies (anti Ia-b and anti ICAM-1 rat anti-mouse (Medac, Hamburg, FRG)) and then followed by secondary antibody (biotynilated goat anti-rat). After washing, avidin-biotin peroxidase complex was used for staining. For negative control primary antibody was substituted with rat serum.

For transmission electron microscopy small cubes from the tail portion of the pancreases were fixed at 4 °C in 2.5% glutaraldehyde in a 0.1M phosphate buffer at pH 7.38, postfixed in 0.1% OsO_4 in the same buffered solution and then dehydrated and embedded in epoxy resins. Uranyl acetate- and lead citrate-stained ultrathin sections (Reichert Ultracut E, Germany) were observed under a Zeiss EM 109 electron microscope (Zeiss, Germany).

Student's t test was used for statistical analysis; the level of significance was set at $p< 0.05$.

Results

Glycaemia was normal in all animals.

There was a remarkable difference in the infiltration of pancreatic ducts located near the islet (islet ducts) and those situated further away (non-islet ducts). Infiltrating elements of islet ducts extended from the duct to its islet. Inflammatory cells also filled postcapillary vessels surrounding these ducts. However, non-islet ducts within the exocrine tissue, as a rule, did not show clear signs of infiltration at the standard light microscopic level; they only showed a peri-ductulitis, i.e. mononucleates within dilated vessels (mainly postcapillary venules) surrounding the ducts.

Immunocytochemistry revealed that pancreatic ducts were strongly positive for both MHC class II (Fig. 1) and ICAM-1 (Fig. 2) antigens and that this positivity was mainly expressed at the level of the connective layer. In particular, ICAM-1 positive cells were mainly located in small and intermediate sized ducts, both in the vicinity and far from the islets. Moreover, elements lining the wall of the ducts showed strong ICAM-1 positivity: these ICAM-1+ cells formed clusters at one pole of the duct or they formed about a half of the entire wall (isolated positive elements were not seen). Furthermore, an ICAM-1 positivity was also found along septa within the exocrine gland (where immunoreactive elements seemed to be dendritic cells) and in vessels (postcapillary venules) close to the ducts (Fig. 2). The maximum expression of ICAM-1 molecules was observed in ducts located near islets in which a peri-islet infiltration or an initial infiltration was seen.

At the electron microscopic level, ducts belonging to animals which did not show clear signs of insulitis did not show infiltrating elements either. Endocrine elements scattered within the epithelial layer usually had the appearance of cholecystokinin (CCK-PZ) elements, due to the presence in their cytoplasm of the typical moderate-dense, medium-sized, round granules and bundles of microfilaments (Fig. 3), or of A cells. In the pancreases whose islets were affected by insulitis,

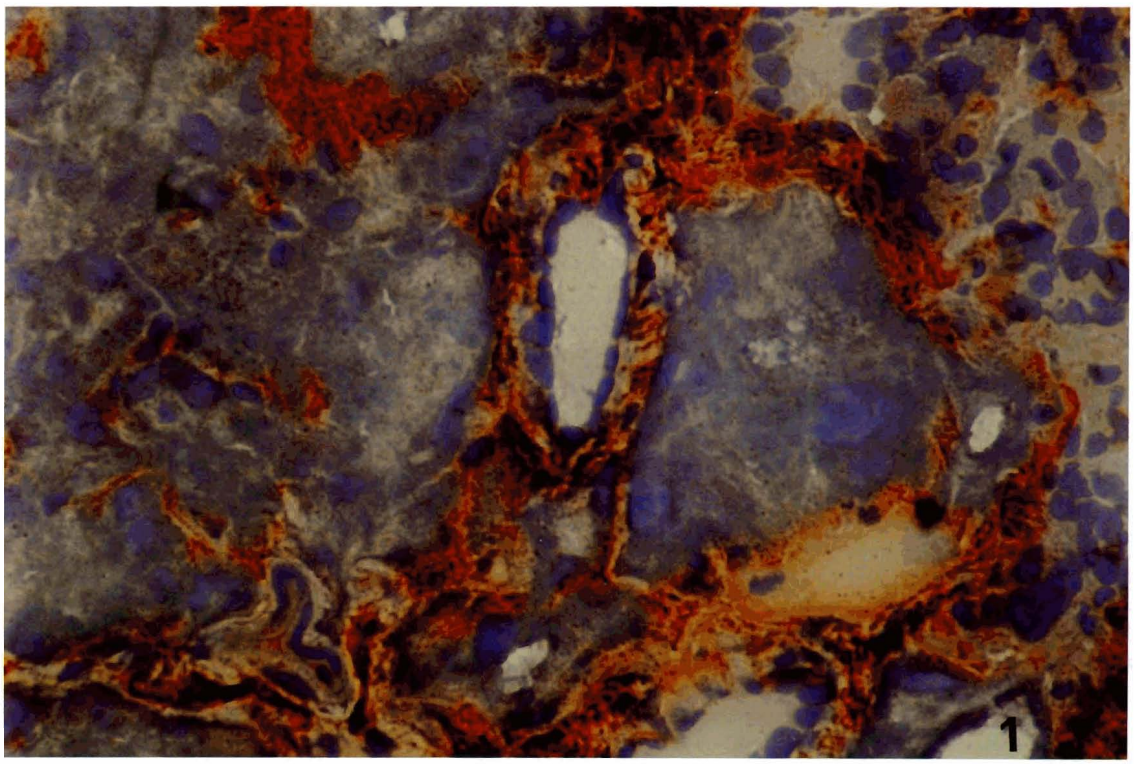

Fig. 1. Light micrograph showing MHC class II immuno-reactivity at the level of a pancreatic islet duct. x 400

single infiltrating elements were also observed in the connective layer of the ducts. These were mainly macrophages or dendritic cells, due to their morphological appearance. Sometimes, these cells were also seen to penetrate the epithelial layer and were detected intermingled with the epithelial cells (Fig. 4). This occurred both in the islet ducts and in the non-islet ducts. Moreover, islet ducts were often surrounded by a periductular infiltrate that extended from the islet infiltrate. Islet ducts were often surrounded by postcapillary venules which were filled with trapped and marginating inflammatory elements.

Discussion

This study has demonstrated that LDS-treated mice show an extra-islet infiltrate of different appearance with respect to that observed in NOD mice (Papaccio et al., 1993a). In the LDS model, only the islet ducts clearly showed infiltration which extended from the vascular pole of the islet to the duct, while non-islet ducts only showed single infiltrating elements which were noticed only at ultrastructural level. However, immunocytochemistry revealed that both islet and non-islet ducts were equally positive for both MHC class II and ICAM-1 antigens. Therefore, the elements expressing these antibodies are not solely infiltrating cells, as these were not present in the non-islet ducts. Most probably this positivity was also expressed by dendritic cells (as these cells are located along septa where immunoreactivity was also noticed) and by endothelia of capillary vessels surrounding these ducts. A strong ICAM-1 positivity demonstrates that adhesiveness is widely represented in early diabetes in this animal model, in which a margination with subsequent transmigration of blood monocytes during the early stages of diabetes has already been described (Papaccio et al., 1991, 1992, 1993b). The reported phenomena were initiated by adhesion of monocytes and other mononucleates to vessel walls, and this was dependent on ICAM-1 expression, a molecule which serves to bind these cells as well as lymphocytes and polymorphonuclear leukocytes to endothelia (Pober and Cotran, 1990). Interestingly, in this animal model, clusters of ICAM-1+ elements were seen within the epithelial lining of the wall of the ductules. This massing of mononucleates is probably due to cytokine secretion with increase ICAM-1 expression and subsequent binding of further mononucleates (Pober and Cotran, 1990).

A major question that should be considered is why does an extra-islet infiltration and, in particular, a «ductular» or «peri-ductular» infiltration occur? Since the anti-islet ß cell attack is a specific autoimmune process that ends with a complete destruction of these elements, what is the aim of this extra-islet infiltration? The existence of other sites of «activation» in the animal models of type 1 diabetes is consistent with the hypothesis of an initially more widespread but less specific process that later undergoes restriction. This concept would also be

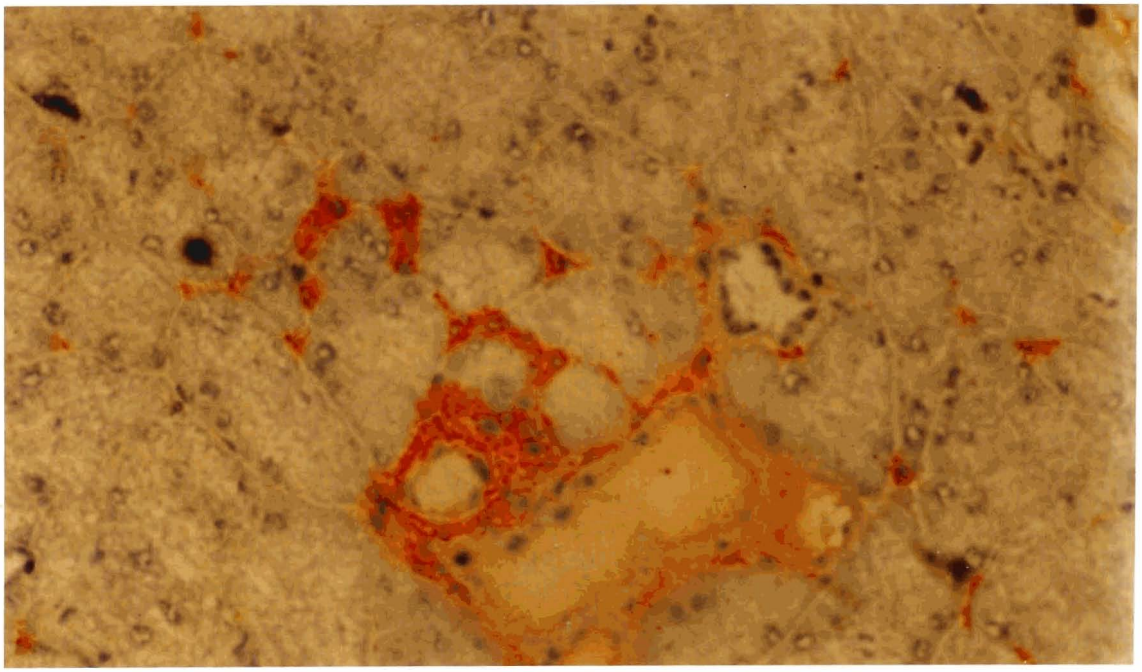

Fig. 2. Light micrograph showing ICAM-1 immunoreactive elements at the level of a pancreatic islet duct. ICAM-1 positivity is also observable on endothelia of a large venule, at the level of the connective layer of a non-islet duct (upper right) and on scattered cells within septa. x 250

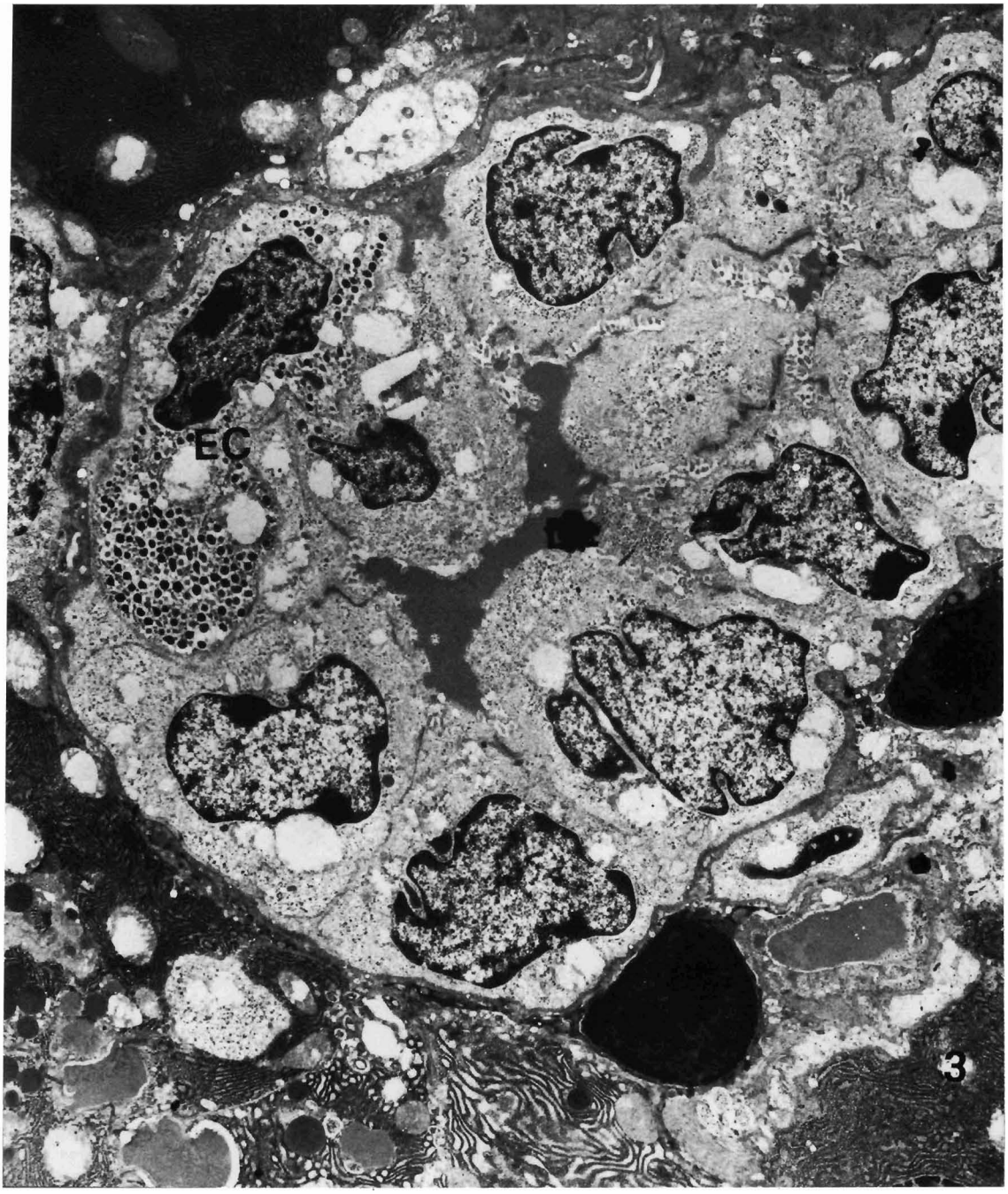

Fig. 3. Transmission electron micrograph of a pancreatic non-islet duct showing an endocrine CCK-PZ element (EC) as part of the epithelial lining. (DL= duct lumen). x 3,000

supported by the appearance of extra-pancreatic infiltration including thyroid, submandibular and Harderian glands, in the NOD mouse (Asamoto et al., 1984; Miyagawa et al., 1986; Sughihara et al., 1989): these organs are all involved in the auto-immune process but they are not destroyed as are the islet ß cells. Why infiltration of extra-pancreatic areas occurs in NOD mice and why infiltration is only limited to intrapancreatic sites (the connective septa of the exocrine pancreas, islets, their ducts and, to a lesser extent, non-islet ducts) in the LDS model (Papaccio et al., 1993a), is not clear and deserves further studies.

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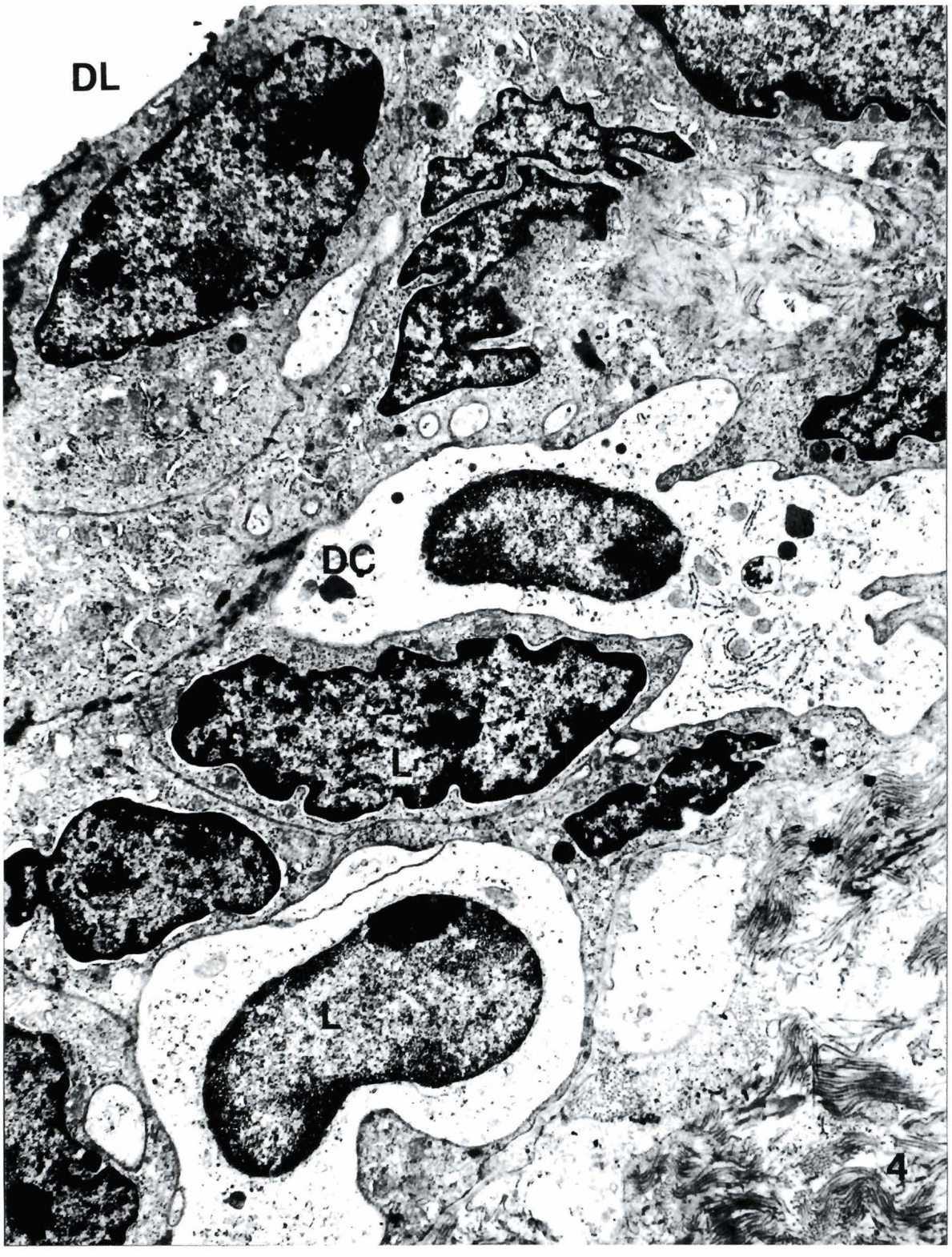

Fig. 4. Transmission electron micrograph showing lymphocytes (L) and dendritic cell (DC) infiltrating a pancreatic duct. DL= Duct lumen. x 3,000

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