ELECTRON MICROSCOPIC STUDIES ON THE INTERACTION OF PANCREATIC ISLET CELLS AND SPLENIC LYMPHOCYTES IN NON-OBESE DIABETIC (NOD) MICE

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ABSTRACT

Lymphocytic infiltration confined to the pancreatic islets is one of the characteristic findings during the diabetic stage of non-obese diabetic (NOD) mice. Electron microscopic observations indicate that lymphocytes come into contact with pancreatic B cells with their villi and sometimes intrude them by cytoplasmic projections. Transmission (TEM) and scanning (SEM) electron microscopy of a mixture of cultured islet cells and T cell-rich splenic lymphocytes from NOD mice revealed a similar manner of contact between both cells in vivo. These observations suggest that immunological mechanisms involving in T lymphocytes may destroy islet cells and lead to the development of overt diabetes in NOD mice.

Non-obese diabetic (NOD) mice are useful animal models for studying insulin-dependent (type I) diabetes mellitus in humans (6, 13, 14). The histopathological characteristic of NOD mice is the infiltration of lymphocytes around and into pancreatic islets (insulitis) beginning at 4-5 weeks after birth. This is observed in more than 90% of male and female NOD mice at 200 days of age. Fujita et al. (1) demonstrated by immunohistochemical and electron microscopic studies that lymphocyte infiltrations in NOD mice were mainly located among the degenerated pancreatic B cells in the islets. On the basis of morphological observations, they suggested that the lymphocytic infiltration selectively and progressively destroy pancreatic B cells, leading to the onset of diabetes in the animal. They thought that the infiltrating lymphocytes might be T cells. In our previous study, the incidence of insulitis could be reduced by a complete thymectomy performed in the neonatal stage of the NOD mice. This supports the view that the pathogenesis of insulitis in NOD mice may be related to the autoimmune mechanisms involved in the defect of the thymus. The purpose of this report is to demonstrate under the electron microscope the interaction between pancreatic B cells and T lymphocytes, paying special attention to the aggressive behavior of the latter against the former and also to reproduce these phenomena in vitro.

MATERIALS AND METHODS

NOD mice were obtained from Aburahi Laboratories, Shionogi & Co. The pancreases of NOD mice at 100 days of age were removed under ether anesthesia, fixed with 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C for 1 h, washed with 0.25 M sucrose, postfixed with 2% OsO4 at 4°C for 1 h, dehydrated with...
an ethanol series, immersed in glycidoxy-\textit{n}-butyl ether and embedded in Epon-812.

For the \textit{in vitro} experiments, the pancreases of 3-week-old NOD mice were used. The pancreatic islets were isolated by digestion with collagenase (4, 12). The suspension of islet cells was prepared by the EDTA-dispase method (9). The cells were cultivated in a culture dish (10 cm in diameter) with RPMI-1640 medium containing fetal calf serum (FCS) and incubated for 7 days at 37°C under 5% CO₂ and 95% air. At the end of the cultivation, the cells were dispersed with RPMI-1640 and resuspended in the same medium. The spleens were also removed from the NOD mice and the non-obese normal (NON) mice which were the normal controls at 100 days of age and their cells were obtained by mashing the spleens on a fine mesh of stainless steel. The cells suspended in the RPMI-1640 medium was filtered through nylon wool (Wako Co.) to concentrate the T lymphocyte population. The cultured pancreatic cells and the T lymphocyte-rich cell suspension were mixed in the suspension at a cell number ratio of 1:10. The mixture was poured into Beem capsules (Beem Co.) and centrifuged at 700 rpm for 5 min. Pellets were further incubated at 37°C for 30 min, and the 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) was poured into each capsule to fix the cell pellet. After keeping the capsules at 4°C for 1 h, the pellets were rinsed with 0.1 M phosphate buffer and fixed again with 2% OsO₄ in the buffer at 4°C for 1 h. They were dehydrated in a graded ethanol series and then divided into two groups. Half of the pellets were immersed in glycidoxy-\textit{n}-butyl ether and embedded in Epon-812. After staining with uranyl acetate and lead citrate, the ultrathin sections of the pancreas previously embedded and the cell pellets were examined with a transmission electron microscope (TEM JEOL, JEM-100CX). The remaining pellets were immersed and unrav-

![Fig. 1 Transmission electron micrograph of lymphocyte of 100-day-old NOD mouse infiltrating around the intra-islet capillary (CP) with cytoplasmic projections (●) protruding into the islet B cells. ×12,000](image-url)
eled in 3-methyl-butyl acetate solution. After critical point drying, they were spatter-coated and examined with a scanning electron microscope (SEM, Hitachi S-450).

RESULTS

Pancreatic islets considered to be in the primary stage of insulitis were obtained from NOD mice and examined by TEM. The lymphocytes had infiltrated around the intra-islet capillaries (Fig. 1) and into the intercellular space of the pancreatic B cells (Fig. 2).

Lymphocytes were frequently in contact with islet B cell membrane via cytoplasmic projections showing broad or pointed contacts. Sometimes, the cytoplasmic projection of lymphocytes intruded deeply into an islet B cell (Fig. 1). SEM observations of the mixtures of splenic lymphocytes and cultured islet cells, revealed many splenic lymphocytes having a few villi in association with islet cells, which were identified by their large size. The villi of the splenic lymphocytes and the cell membrane of the islet cells seemed to be in close contact (Fig. 3). The contact sites of these cells were examined also by TEM. Splenic lymphocytes were seen with many cytoplasmic projections containing microfilaments protruding into the islet cells. Some of these projections were in broad contact with the islet cell membrane showing an electrondense material between the cell membranes, while others revealed points of contact (Fig. 4). Occasionally, some projections intruded deeply into the cytoplasm of the islet cells, as seen in vivo (Fig. 5).

Normal control lymphocytes from NON mice, sister strain of NOD mice, did not show such intimate contact with islet cells.

DISCUSSION

Fujita et al. (1) reported that specific infiltra-

Fig. 2  The same situation as in Fig. 1. Lymphocytes infiltrate the intercellular space of islet B cells.  \times7,200
tion of lymphocytes into the B cells of pancreatic islets was produced selectively by degeneration of the B cells, and this was a morphological characteristic of the NOD mice. Based on the ultrastructural observations, they also suggested that the lymphocytes infiltrating the islets of NOD mice were T cells. In the present study, the splenic lymphocytes were in close contact with the pancreatic islet cells with cytoplasmic projections after mixing and culturing with cells derived from NOD mice in vitro, but not when mixed with control lymphocytes from NON mice.

The interactions between lymphocytes and islet cells observed in the present study were similar to those reported previously between effector lymphocytes and target cells (2, 10). The same interactions were observed in vivo between infiltrating lymphocytes and islet B cells in NOD mice at about 100 days of age. These results suggest that a lymphocytic cell-mediated cytotoxic mechanism may be involved in the phenomenon of the insulitis in NOD mice. Sanderson (10) has reviewed the
mechanism of lymphocyte-mediated cytotoxicity and suggested three kinds of effectors for the appearance of cytotoxicity: 1) killer T cells derived from the thymus, 2) antibody-dependent killer (K) cells and 3) natural killer (NK) cells, showing similar and basic mechanisms for killing, long cytoplasmic projections from these effector cells and receptor-ligand interaction. Our results obviously support the possibility that the cytotoxicity of T cells derived from the thymus is involved in the insulitis of NOD mice, since the cells in contact with islet cells did not contain granules, which are characteristic of the large granular lymphocytes (LGL i.e. NK cell) (11). On the other hand, Miyazaki et al. have found that cytotoxic or suppressor T cells were increased in the spleen and the sites of insulitis of the NOD mouse at the initial stage of insulitis (7), Ogawa et al. (8) have reported a reduction of insulitis by neonatal thymectomy. It was also reported that NOD mice to which the gene of athymic nude mice was introduced did not develop insulitis (3), and that the NOD mouse had rather low NK activity (15). Some investigators have reported that not killer but helper T cells might be the dominant cell population of insulitis on NOD mice and suggested T cell-mediated cell cytotoxicity might not play an essential role in the occurrence of diabetes in NOD mice (5, 16). However, since the dominant cell population does not always play an essential role in the immuno-reaction, it can not be disproved that T cell-mediated cell cytotoxicity plays an essential role in the onset of insulitis of NOD mouse. To determine the cell type of the lymphocytes protruding into the cytoplasmic projections and interacting with islet B cells, and to find the cytotoxicity of each subset of lymphocytes, ultrastructural
immunohistochemistry and in vitro study are needed. The NOD mouse can be a useful animal model for studying not only the etiology of type I diabetes mellitus but also the mechanisms of cell-mediated cytotoxicity.

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REFERENCES


