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THE ROLE OF IMMUNOHISTOCHEMISTRY IN PANCREATIC ENDOTHELIUM IN DIABETES MELLITUS

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Abstract:
We examined pancreas biopsy specimens from 18 newly diagnosed insulin-dependent diabetes mellitus (IDDM) patients to elucidate the mechanism underlying beta cell destruction. Pancreas islets were seen in all patients and insulitis in eight patients. Infiltrating mononuclear cells consisted of CD4+T, CD8+T, B lymphocytes, and macrophages. Among them, CD8+T lymphocytes were predominant and macrophages followed. The expression of MHC class I antigens was increased in islet and endothelial cells in nine patients. MHC class II expression was increased in endothelial cells of the same patients. The expression of intercellular adhesion molecule-i was increased in endothelial cells in two of the nine patients with MHC hyperexpression; in one of them, lymphocyte functionassociated antigen-3 expression was also increased.

Keywords: immunohistochemistry, pancreatic endothelium, diabetes mellitus.

Introduction

It is interesting to investigate the expression of adhesion molecules, intercellular adhesion molecule-I (ICAM-l), and lymphocyte function-associated antigen-3 (LFA-3). These molecules have recently been shown to play a crucial role in both cell-cell adhesion and activation in immune response. ICAM-l can be expressed on a wide variety of cells in response to inflammatory cytokines and bind to lymphocytes and monocytes through their cell surface LFA- 1. LFA-3 is ubiquitously distributed and binds to CD2 on T lymphocytes. Although the ICAM-1/LFA-1 and LFA-3/CD2 interactions might participate in the process of beta cell destruction, there has been no systematized study on the expression of adhesion molecules in IDDM pancreases. We have reported that pancreas biopsy under laparoscopy is a safe procedure to obtain pancreas tissues from newly diagnosed IDDM patients who survived acute metabolic disorders. This procedure has the advantage in dissecting ongoing

immunopathological events in pancreases and correlating revealed immunopathological findings to the clinical aspects of patients, as compared with other autopsy studies. Using pancreas biopsy specimens from newly diagnosed IDDM patients, we examined mononuclear cell infiltration into the islet, the phenotype of infiltrating cells and the expression of cell surface molecules, MHC antigens, ICAM-1, and LFA-3 to address above mentioned issues and elucidate the mechanism underlying the beta cell destruction in IDDM. We also analyzed the relation between these findings and the clinical characteristics of the patients.

The study protocol described below was approved by the ethical committee of Osaka University Medical School and was carried out in accordance with the Declaration of Helsinki. The purpose of the study was to closely examine IDDM pancreases as soon as possible after disease onset to elucidate the pathogenesis of IDDM. The method of pancreas biopsy was previously described. Briefly, a laparoscope was inserted into the abdominal cavity under local anesthesia. After direct inspection of the pancreas, a piece of tissue was obtained from the body of the pancreas with a biopsy forceps. Special attention was paid not to injury any visible vessels during biopsy and to confirm clotting at the biopsy site after biopsy. Possible complications were allergic reaction, pancreatitis, peritonitis, bleeding, and so on. We explained the purpose, method, risks, and the freedom of consent to the patients and their families, and obtained written informed consent from all ofthem. In all patients, pancreas biopsies were performed without any complications.

We revealed mononuclear cell infiltration into the islet in newly diagnosed IDDM patients who survived acute metabolic disorders. The use ofbetter antibodies coupled with an avidinbiotin system enabled us to detect insulitis even in the cases in which insulitis had not been observed in our earlier examination. However, both of the number of infiltrating cells and the proportion of patients with insulitis seemed to be fewer than that observed in autopsy studies reported in Europe. Several possible explanations exist for this difference. The severity of metabolic disorders might affect the function of mononuclear cells and subsequently immune response against beta cells. The age ofthe patients might be related to the degree of immune response. Since the frequency of insulitis was reported to be high in recent-onset IDDM patients under 15 yr of age, the higher age of the patients in our study could explain the difference in the frequency and the degree of insulitis. Racial difference also has to be considered. Since HLA types susceptible to IDDM in Japanese are different from those in Caucasians, immune response against beta cell autoantigens might be different between the two ethnic groups. The time of biopsy is another important factor. Although the time of biopsy was only 3 mo on an average after clinical onset of IDDM,

the fact that the volume of beta cells was markedly decreased to less than one fifth of that in control subjects in newly diagnosed patients suggests that the cell-mediated immune response is already in a late stage. Finally, we have to consider a sampling problem. Autopsy studies on the whole pancreas indicated the heterogeneous distribution ofthe pathological region. Considering this issue, we have always taken biopsy samples from the same position of the pancreas to decrease variation among the patients. In addition, we have always examined sections from several different parts of the biopsy tissue. We analyzed the composition of mononuclear cells infiltrating into the islet by the double IFL method using diverse monoclonal antibodies. CD8 + Tlymphocytes were the predominant subpopulation and macrophages followed. Fewer CD4+T and B lymphocytes were also seen. This composition of infiltrating cells was comparable with that observed in a previous autopsy case and pancreas-transplanted cases. Very recent pathological study on the autopsy pancreas at the onset ofIDDM also gave the same finding. To date, all the cases in which phenotypic analyses of infiltrating cells could be performed subsequently showed that CD8+T lymphocytes were the predominant subpopulation. CD8 + T lymphocytes recognize target antigens in conjunction with self MHC class I molecules and act as the chief effector in cellular immunity.

Macrophages, CD4+T and B lymphocytes are other subpopulations observed in the islet in our series. Although the crucial role of macrophages in the pathogenesis of IDDM is recognized by the fact that the impairment of macrophage function prevents diabetes in animal models it remains to be elucidated in humans. We and others showed that some parts of infiltrating cells were certainly macrophages. It is conceivable that macrophages participate in the process of beta cell destruction by initiating immune response as antigen-presenting cells, by producing cytokines or by phagocytosis of damaged beta cells. CD4+ lymphocytes are other candidates for effectors in autoimmune beta cell destruction. CD4+T lymphocytes might act as helper cells in autoimmune response against beta cells. Sequential studies ofthe pancreas of T cell-transferred NOD mice demonstrated that CD4 (L3T4)+ lymphocytes predominated at an early phase ofinsulitis and CD8 (Lyt-2)+ lymphocytes increased at a later stage. However, it has not been clear what mononuclear cells are major constituent of infiltrating cells at an initial stage of insulitis in human, since human pancreases could not be analyzed in prediabetic period. CD8+ lymphocytes are possibly predominant from the early phase ofinsulitis in human IDDM as seen in our study. This is supported by the finding that CD8 + lymphocytes were already main infiltrating cells into islets of grafted pancreas when beta cells were relatively preserved and diabetes had not recurred.

We found the close relation between insulitis and MHC class I antigen hyperexpression as mentioned. There has been controversy as to which is the first event and it could not be clarified even in our study. If the hyperexpression of MHC class I antigens occurs first, it would elicit autoreactive T lymphocyte infiltration, and if the converse is the case, infiltrating lymphocytes would provoke cytokine production resulting in the hyperexpression of MHC class I antigens in islet cells. Irrespective of the order, both of these phenomena seem to accelerate the cascade of autoimmune response in IDDM pancreases. In relation to these phenomena, it is interesting to determine whether cytokines are expressed in islet cells or infiltrating mononuclear cells as reported by Foulis et al since cytokines are important factors in enhancing autoimmune response. Therefore, we examined the expression of IFN-a, IFN-'y, IL-l and IL-6, but could not find the expression of these cytokines in any cells of IDDM pancreases (unpublished observation). This might be caused by the poor sensitivity of used antibodies and could be clarified by more sensitive methods such as in situ hybridization.

In conclusion, we revealed that insulitis is predominantly composed of CD8+T lymphocytes and that there is a close relationship between CD8 +T lymphocyte-predominant insulitis and MHC class I antigen hyperexpression in islet cells in the pancreas biopsy specimens from newly diagnosed IDDM patients. This suggests that CD8+T lymphocytes play a significant role in the pathogenesis of IDDM via the recognition of autoantigens in association with hyperexpressed MHC class I molecules. Although the hyperexpression of adhesion molecules was observed in two patients, its role in immune response in IDDM still remains to be elucidated. Our findings might not reflect the very first events of the disease. However, these results could not be elicited from other studies using animal models or human autopsies.

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