CD4⁺ T cell–dominant insulitis in acute-onset Type 1 diabetes mellitus associated with intraductal papillary mucinous adenoma

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Abstract. The loss of insulin-producing pancreatic β-cells in Type 1 diabetes mellitus (DM) is presumably the result of a T cell–mediated process. In general, CD8⁺ T cells are the predominant lymphocytes in the insulitis lesions, and CD4⁺ T cell–dominant insulitis is very rare. We present a case of a 72-year-old woman presented with excessive thirst and a 3-month history of weight loss. She was in a state of ketosis, and her plasma glucose concentration and HbA1c value were elevated. Moreover, anti-islet autoantibodies were positive, thus acute-onset Type 1 DM was diagnosed. At the time of diagnosis, a tumour was detected in the pancreas; total pancreatectomy was carried out 2 months later. The pathological diagnosis was intraductal papillary mucinous adenoma. Immunohistochemical staining of a sample of non-tumorous pancreatic tissue revealed 13 insulitis lesions infiltrated by both CD4⁺ and CD8⁺ T cells, and interestingly there were more CD4⁺ T cells than CD8⁺ T cells in the lesions. Moreover, B cells and macrophages had also infiltrated the lesions, and these two cell frequencies were both positively correlated with CD4⁺ as well as CD8⁺ T cell frequencies. This was a rare case with acute-onset Type 1 DM characterized by CD4⁺ T cell-dominant insulitis. Proinflammatory cytokines that can promote β-cell apoptosis or CD8⁺ T cell function are reported to be secreted from CD4⁺ T cells. Thus, together with B cells and macrophages, CD4⁺ T cell–associated immune responses may have, directly and/or indirectly, played a role in the pathogenesis of the Type 1 DM in this patient.

Key words: Type 1 diabetes mellitus, CD4⁺ T cell, Insulitis, Pathogenesis

Case Report

A 72-year-old woman presented at our hospital with excessive thirst and a 3-month history of weight loss. Her plasma glucose concentration was 18.7 mmol/L (337 mg/dL) and her HbA1c value was 11.7% (104 mmol/mol); both measurements had been normal at a health check 9 months previously. Additional lab test results were as follows: urine ketone, 3+; urine C-peptide, 2.4 µg/day; GAD antibody (RSR, UK), 1.8 U/mL (normal value, < 1.3 U/mL); and insulinoma-associated protein 2 antibody (RSM), 2.3 U/mL (normal value, < 0.5 U/mL). On the basis of these results, acute-onset Type 1 DM was diagnosed. The patient had HLA-DR9, which confers susceptibility to Type 1
DM in Japanese people. At the time of diagnosis, a tumour (48 mm in size) suspicious of malignancy was detected in the head of the pancreas (Fig. 1); 2 months later total pancreatectomy was performed. The pathological diagnosis was intraductal papillary mucinous adenoma (IPMA).

To investigate the histological changes in insulitis (after obtaining informed consent), two different samples of non-tumorous pancreatic tissue obtained from a normal region of the tail of the pancreas (Fig. 1) were subjected to haematoxylin and eosin staining and immunohistochemical staining for insulin, CD4, CD8, CD79a (a key component of B cell antigen receptor complex), CD68, major histocompatibility complex (MHC) class I and forkhead box protein 3 (Foxp3) (Fig. 2). Complete or partial brown staining of the circumferential linear cell surface membrane at any intensity was defined as positive staining for CD4, CD8, CD79a and CD68, and brown nuclear staining of these cell surface markers, for reasons unknown, was not considered as positive staining. According to a previous report [2], we defined the islets with five or more infiltrating mononuclear cells as insulitis-positive ones.

A light microscopic examination of haematoxylin and eosin sections identified a total of 41 islets, many of which were small and atrophic. As a result, we identified 13 insulitis-positive islets, and found that there were more CD4+ T cells than CD8+ T cells which had infiltrated the lesions (84.7 ± 36.5 CD4+ T cells/islet versus 45.8 ± 27.2 CD8+ T cells/islet) (Table 1). Indeed, 12 out of 13 insulitis lesions (i.e., the exception being islet No.4) showed CD4+ T cell-dominant insulitis.

In addition, CD79a+ cells (B cells) and CD68+ cells (macrophages) also infiltrated the insulitis lesions (Table 1). CD79a+ cell frequency was significantly positively correlated with CD4+ T cell (Pearson’s correlation coefficient ($r_p = 0.754$, $P < 0.01$) and CD8+ T cell frequencies ($r_p = 0.687$, $P < 0.01$). Moreover, CD68+ cell frequency was also significantly positively correlated with CD4+ T cell ($r_p = 0.818$, $P < 0.01$) and CD8+ T cell frequencies ($r_p = 0.770$, $P < 0.01$). Meanwhile, we detected only a small number of immune cells expressing Foxp3, a marker of immunoregulatory T cells, suggesting only a limited role for immune regulation in insulitis lesions (Table 1). Foxp3+ cell frequency was not significantly correlated with CD4+ T cell and CD8+ T cell frequencies.

Finally, MHC class I antigen was expressed to some extent throughout almost all islets with insulitis. Some of the cells expressing this antigen were characterized by a high nuclear/cytoplasmic (N/C) ratio, suggesting that these cells were believed to be lymphocytes infiltrating the insulitis lesions (arrows in Fig. 2h).

**Discussion**

Type 1 DM is caused by a significant loss of insulin-producing pancreatic β-cells, presumably as the result of a T cell–mediated process; insulitis is a feature of Type 1 DM that may contribute to β-cell destruction [1]. The histopathological analysis of samples of pancreatic tissue collected around the time of diagnosis of Type 1 DM generally shows infiltration of T cells, B cells, and macrophages into islets [2, 3]. In a previous study of pancreas samples from younger patients (mean age, 11.7 years) with recent-onset Type 1 DM, CD8+ T cells were found to be the predominant lymphocytes in the inflammatory infiltrate within islets, and CD4+ T cells were less abundant than either CD8+ T cells or macrophages [2]. Moreover, infiltration is accompanied by a marked upregulation of MHC class I antigen on islet cells [3]. These findings suggest that CD8+ T cells are the dominant effector cells in Type 1 DM.
Fig. 2  Representative images showing the histology of insulitis lesion (islet No.8) in non-tumourous pancreatic tissue from a 72-year-old woman. Haematoxylin and eosin staining (a) and immunohistochemical staining of insulin (b), CD4 (c), CD8 (d), CD79a (e), CD68 (f), Foxp3 (g) and MHC class I (h). (c) Arrows show three representative CD4+ T cells. (g) Foxp3+ cells can be identified by the brown nuclear staining with anti-Foxp3 antibody. Arrows show three representative Foxp3+ cells. (h) Arrows show three representative cells with a high N/C ratio (very little cytoplasm), which were characterized by the expression of MHC class I antigen on the cell surfaces. These cells are believed to be lymphocytes infiltrating the insulitis lesions. Immunohistochemical staining was performed using the following first antibodies: guinea pig polyclonal anti-insulin (Dako, Glostrup, Denmark); rabbit polyclonal anti-glucagon (Dako); CD4 antibody, clone 1F6 (Nichirei, Tokyo, Japan); CD8 antibody, clone C8/144B (Nichirei); CD68 antibody, clone KP1 (Dako); CD79a antibody, clone JCB117 (Dako); Foxp3 antibody, clone 236A/E7 (Abcam, MA, USA); MHC class I antibody, clone EP1395Y (Novus Biologicals, CO, USA). Scale bar = 100 µm.
Moreover, CD4+ T cells may also help to promote CD8+ T cell functions through the secretion of IL-21. In non-obese diabetic (NOD) mice, a model of human Type 1 DM, IL-21 is reported to be required for the development of autoimmune diabetes [8, 9], and deficiency in IL-21 signaling prevents insulitis and diabetes via alteration in CD8+ T cell function within the islets [8]. Moreover, a recent study revealed that IL-21 promotes cytotoxic CD8+ T cell function and killing of pancreatic islets, contributing to the development of Type 1 DM [10]. Considering that Ferreira et al. demonstrated the increased frequency of IL-21-producing CD4+ effector memory T cells in patients with Type 1 DM [11], CD4+ T cells might also take a facilitating role in the development of human Type 1 DM through IL-21 secretion. This may be true of our patient as well.

Recently, Arif et al. reported that peripheral blood CD4+ T cells from patients with Type 1 DM secrete IL-17 in response to β-cell autoantigens [12]. In addition, CD4+ T cells primed by an antigen-presenting cell (APC) can activate the APC through CD40–CD40-ligand (CD40L) interactions, and the activated APC can then promote the CD8+ T-cell responses, generating cytotoxic effector CD8+ T cells [7]. These activated Th1 CD4+ T cells and cytotoxic CD8+ T cells are probably involved in the destruction of β-cells.

<table>
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<th>No. of islets with insulitis</th>
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<td>106</td>
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Average (mean ± SD) 84.7 ± 36.5 45.8 ± 27.2 53.8 ± 28.4 17.6 ± 7.2 13.0 ± 9.2

| Ratio of each cell to CD4+ T cells | 1.0 | 0.59 ± 0.28 | 0.62 ± 0.30 | 0.23 ± 0.08 | 0.16 ± 0.09 |

Each immune cell number is expressed as the cell number per islet with insulitis. Two independent observers, blind to the immune cell type, counted the cell number and the results are shown as the average of the two values and the mean ± standard deviation (SD). CD4, CD4+ T cells; CD8, CD8+ T cells; CD79a, CD79a+ B cells; CD68, CD68+ cells (macrophages); Foxp3, Foxp3+ immunoregulatory T cells.

To our knowledge, CD4+ T cell–dominant insulitis is very rare, and the histological picture has not been photographically recorded, at least not in acute-onset Type 1 DM, with the exception of our previously reported case of latent autoimmune DM in adults (LADA) [4]. In the present patient, the greater age at onset of Type 1 DM may be associated with the dominance of CD4+ T cells, for reasons unknown. Proinflammatory cytokines [e.g. tumour necrosis factor-α (TNF-α), interferon-γ and interleukin (IL)-1β] that can promote β-cell apoptosis are secreted not only from macrophages and dendritic cells but also from CD4+ T cells [5], possibly contributing to the development of Type 1 DM.

A previous study indicated that IL-2 released from activated T helper 1 (Th1) CD4+ T cells can activate diabetogenic CD8+ T cells, which subsequently differentiate into cytotoxic CD8+ T cells and infiltrate the pancreatic islets [6]. In addition, CD4+ T cells primed by an antigen-presenting cell (APC) can activate the APC through CD40–CD40-ligand (CD40L) interactions, and the activated APC can then promote the CD8+ T-cell responses, generating cytotoxic effector CD8+ T cells [7]. These activated Th1 CD4+ T cells and cytotoxic CD8+ T cells are probably involved in the destruction of β-cells.

Moreover, CD4+ T cells may also help to promote CD8+ T cell functions through the secretion of IL-21. In non-obese diabetic (NOD) mice, a model of human Type 1 DM, IL-21 is reported to be required for the development of autoimmune diabetes [8, 9], and deficiency in IL-21 signaling prevents insulitis and diabetes via alteration in CD8+ T cell function within the islets [8]. Moreover, a recent study revealed that IL-21 promotes cytotoxic CD8+ T cell function and killing of pancreatic islets, contributing to the development of Type 1 DM [10]. Considering that Ferreira et al. demonstrated the increased frequency of IL-21-producing CD4+ effector memory T cells in patients with Type 1 DM [11], CD4+ T cells might also take a facilitating role in the development of human Type 1 DM through IL-21 secretion. This may be true of our patient as well.

Recently, Arif et al. reported that peripheral blood CD4+ T cells from patients with Type 1 DM secrete IL-17 in response to β-cell autoantigens [12]. In addition, they showed that IL-17 is actively produced in inflamed islets close to the onset of disease and provided evidence for the existence of a pathway for β-cell destruction via IL-17. Thus, CD4+ T cells secreting IL-17, that is Th17 cell, may also contribute to Type 1 DM as effector cells [13]. Taken together,
in addition to the cytotoxic effects of CD8\(^+\) T cells, CD4\(^+\) T cell–associated immune responses may also have played a role in the pathogenesis of Type 1 DM in this patient.

CD4\(^+\) T cells generally display not only a Th1, but also a regulatory phenotype; i.e., CD4\(^+\) immunoregulatory T cells (Tregs). It was reported that the progression of insulitis is associated with a progressive reduction in the ratio of Tregs to effector T cells within islets in NOD mice [14]. On the other hand, there has been no report on the population of Tregs infiltrating insulitis lesions of human Type 1 DM. Although whether intra-islet Tregs can control the progression of insulitis or not remains to be confirmed, the very low frequency in Foxp3\(^+\) regulatory T cells in insulitis lesions suggests that there is little involvement of any immune regulation in the lesions and only partial contribution to the promotion of anti-islet autoimmunity in the present patient. To clarify the details, further study will be necessary.

As mentioned above, insulitis is generally accompanied by a marked upregulation of MHC class I antigen on the surface of islet cells under immune attack; this finding is considered to be a typical hallmark of autoimmune Type 1 DM [3, 15]. Considering that autoreactive cytotoxic CD8\(^+\) T cells recognize peptide antigens presented on the β-cell surface by MHC class I antigen and destroy the β-cells [16], in the present patient the lower frequency in CD8\(^+\) T cells infiltrating islets both with or without β-cells and produce inflammatory cytokines in patients with Type 1 DM [2, 17]. In our case, the CD68\(^+\) cell number was positively correlated with CD4\(^+\) as well as CD8\(^+\) T cell frequencies, macrophages might be contributing to not only the activation of β-cell toxic CD8\(^+\) T cells, but also the generation of autoreactive CD4\(^+\) T cells.

Dendritic cells (DCs) possibly play an important role in the development of Type 1 DM. Uno et al. revealed that DCs as well as macrophages infiltrated the islets both with or without β-cells and produce inflammatory cytokines in patients with Type 1 DM [2]. However, we had no opportunity to stain CD11c, a marker of DCs, in this study. As a matter of fact, we performed immunohistochemical staining according to the previous report of Willcox et al. [2], in which CD11c\(^+\) cell was not investigated, to compare our findings to theirs in this report. Because DCs are reported to have strong antigen-presenting activity in mice and humans [23], there might be a certain relationship between the frequency of DCs and other immune cells including CD4\(^+\) T cells in this case. In future, we should plan to keep an eye on DCs infiltrating islets in Type 1 DM.

The reason for the rare appearance of CD4\(^+\) T cell–dominant insulitis in usual Type 1 DM remain to be elucidated in this report. The earlier study revealed that CD8\(^+\) T cells infiltrating islets peaked according to the number of remaining functional β-cells and rapidly disappeared when all functional β-cells were destroyed. Meanwhile, the infiltrating CD4\(^+\) T cell number was much less variable during the period of β-cell decline [2]. These findings suggested the possible existence of the moment when CD4\(^+\) T cells more than CD8\(^+\) T cells, few if any, may temporarily infiltrate islets especially in the latest stage of disease process. As for this case as well, the disease process might already have been in the latest stage even just 2 months after the disease onset, leading to the appearance of CD4\(^+\) T cell-
dominant insulitis. In fact, it is generally considered that cross-sectional histological studies around the time of diagnosis of Type 1 DM represent a picture of the final stages of disease [24]. Considering the importance of the timing of histological examination, we should collect and investigate similar cases at different times after the disease onset in future.

Whether the existence of IPMA might affect our distinctive histological findings or not remains to be elucidated due to a lack of similar cases. Intriguingly, a recent case report of intraductal papillary mucinous neoplasm (IPMN) without Type 1 DM demonstrated that increased CD4+ T cell, CD8+ T cell and CD68+ cell infiltrations were seen in the stroma surrounding areas with high-grade dysplasia compared to areas with intermediate-grade dysplasia. Meanwhile, little to no T cell and B cell infiltration was detected in the stroma surrounding adjacent normal epithelia [25]. These findings suggested that, if there is a sufficient distance between the insulitis and IPMA lesions, the insulitis lesion may not be histologically affected by IPMA, and this is believed to be true of our patient as well. However, we have no evidence that IPMA does not absolutely affect the histologic findings of adjacent insulitis lesions, and have to admit the possibility of a certain connection between CD4+ T cell–dominant insulitis and adjacent IPMA. To clarify the details, we need to collect and investigate similar cases in future.

The present study has several limitations. First, this is a single case report and the aetiology of the distinct histopathological findings could not be fully explained or inferred due to the lack of similar cases. Second, as previously reported, a variable degree of insulitis may be seen in different regions of the pancreas of one patient with newly diagnosed Type 1 DM, which means that the islets in one pancreatic lobule may appear normal, while those in adjacent lobules may be small or have profound insulitis [26, 27]. Therefore, whether the variability in the degree and/or characteristics of insulitis in the pancreas affect the proportion of immune cells infiltrating islets or not should have been evaluated in this patient. However, we did not have any chance to conduct a detailed survey due to the limitation on the use of the tissue block.

Conclusions

We present a case of acute-onset Type 1 DM associated with IPMA characterized by CD4+ T cell–dominant insulitis, which may provide support for a role of CD4+ T cell–associated immune responses in the pathogenesis of Type 1 DM. The pathogenic distinction between CD4+ and CD8+ T cell–dominant insulitis should be elucidated through investigation of similar cases.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References


