Protective role for cytosolic phospholipase A2α in autoimmune diabetes of mice


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1. Introduction

The non-obese diabetic (NOD) mouse, an excellent model for human type 1 diabetes, spontaneously develops autoimmune diabetes [1,2]. In this model, insulitis is caused by the infiltration of macrophages as well as CD4+ and CD8+ T cells [3], and the presence of cytokines such as tumor necrosis factor (TNF)-α in the insulitis lesion is implicated in the development of diabetes [4]. It is also well known that macrophage plays a crucial role in the progression of insulitis and development of diabetes.

Arachidonic acid is liberated from membrane phospholipids mainly by phospholipase A2 (PLA2). Macrophages contain at least three different PLA2: i.e., a cytosolic phospholipase A2α (cPLA2α) and a secretory PLA2 (sPLA2), both of which require Ca2+ ion, and a Ca2+ ion-independent PLA2 (iPLA2) [5,6]. Several studies have demonstrated that some cPLA2α-mediated eicosanoids are involved in the immune system [6].

In particular, prostaglandin E2 (PGE2), a cyclooxygenase (COX) product of cPLA2α-mediated arachidonic acid, is a major eicosanoid released by antigen-presenting cells (APCs). Therefore, it can be expected that cPLA2α-mediated eicosanoids play significant roles in autoimmune diseases, including type 1 diabetes. Previously, we have established cPLA2α knockout mice that showed a marked decrease in the production of eicosanoids by their peritoneal macrophages [7]. In this study, we established cPLA2α knockout NOD mice to investigate the role of cPLA2α in the autoimmune responses during the development of diabetes.

2. Materials and methods

2.1. Mice

cPLA2α knockout NOD mice were obtained by crossing cPLA2α knockout C57BL/6 mice [7] with NOD mice (CLEA Japan, Tokyo, Japan). In the N6 generation, the male that was the most enriched for NOD-type microsatellite markers was selected by the speed congenic approach as described elsewhere [8]. We found a male mouse in that generation that had NOD-type microsatellite markers in all its chromosomes, and it was used to produce the next generation. The cPLA2α+/− (heterozygous knockout) NOD mice in the N11 generation were then intercrossed to generate cPLA2α+/− (wild-type), cPLA2α+/−, and cPLA2α−/− (homozygous knockout) female NOD mice. Mice were kept under specific pathogen-free conditions in the animal facility of Osaka University Graduate School of Medicine. Urinary glucose analysis was performed using Tes-tape (Eli Lilly, Indianapolis, IN) weekly starting at 10 weeks of age. Plasma glucose levels were determined using Glutest-Ace (Sanwa Kagaku, Nagoya, Japan) when glucosuria was detected, and mice were considered to be diabetic after two consecutive examinations of blood glucose values >250 mg/dl.

2.2. Genotype analysis by PCR

The genotype of the cPLA2α locus was determined by PCR analysis using genomic DNA prepared from tail biopsies as the template. The nucleotide sequences of the primers were described elsewhere [7]. The reaction was performed using a Taq polymerase (Life Technologies, Grand Island, NY). The PCR was performed at 94 °C for 2 min, and then 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by extension at 72 °C for 10 min. The PCR products were separated by electrophoresis in a 1.5% agarose gel.

2.3. Histology

The pancreas was removed from each mouse, fixed in 10% formol-hyde, and embedded in paraffin. Thin sections at five levels, 100 μm apart, were cut for staining with hematoxylin-eosin to evaluate the islet-infiltrating immune cells by light microscopy. At least 25 islets from each mouse were observed and scored by two independent blinded observers using the following criteria: grade 0, islets free of insulitis;

Abbreviations: APC, antigen-presenting cell; cPLA2α, cytosolic phospholipase A2α; FBS, fetal bovine serum; NO, nitric oxide; NOD, non-obese diabetic; PGE2, prostaglandin E2; ROS, reactive oxygen species; TNF, tumor necrosis factor

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grade 1, islets with lymphocyte infiltration in <25% of the area; grade 2, lymphocyte infiltration in 25–50% of the area; grade 3, lymphocyte infiltration in 50–75% of the area; grade 4, lymphocyte infiltration in >75% of the area or small retracted islets. The histological score gives the mean infiltration grade of the islets analyzed.

2.4. Cytokines and PGE2 measurement by ELISA
Mouse IL-12, mouse TNF-α, and PGE2 levels were analyzed using a mouse IL-12 ELISA kit (Endogen, Woburn, MA), mouse TNF-α ELISA kit (Endogen) and PGE2 ELISA kit (Amersham Biosciences, Buckinghamshire, UK), respectively, according to the manufacturer’s instructions.

2.5. Preparation and stimulation of peritoneal macrophages
The procedures were published previously [7]. Briefly, peritoneal exudate cells were harvested by injecting 10 ml of ice-cooled phosphate-buffered saline (PBS) into the peritoneal cavity. The collected cells were transferred to each well of a flat-bottom, 96-well microplate at 3 x 10^6 cells/200 μl in RPMI1640 medium (Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies) and penicillin/streptomycin (Life Technologies). The adherent cells after 1 h of incubation were used as resident peritoneal macrophages. After 18 h incubation, the medium was aspirated, replaced with FBS-free RPMI1640 medium containing the Ca^{2+} ionophore A23187 (5 μM; Wako Pure Chemical Industries, Osaka, Japan) or LPS (1 μg/ml; Sigma) and incubated for 30 min or 8 h, respectively. After the supernatants were collected, IL-12, TNF-α, and PGE2 were quantified using the ELISA kit described above.

2.6. Statistical analysis
Results are presented as the means ± SE. The log-rank test was used to compare the incidence of diabetes. Differences in insulin score and cytokine production levels were analyzed using the Mann–Whitney U test. A value of P < 0.05 was considered statistically significant.

3. Results and discussion
As shown in Fig. 1, the cumulative incidence of diabetes was significantly higher in the cPLA2α−/− NOD mice than in the cPLA2α+/+ or cPLA2α+/− NOD mice, although there was no difference in the timing of the onset of diabetes among the three groups. The mean insulin score was significantly higher at 18 weeks of age (diabetic stage; i.e., just before the onset of overt diabetes) in the cPLA2α−/− NOD mice than in the cPLA2α+/+ and cPLA2α+−/− NOD mice, although no significant difference in insulin scores was observed at 10 weeks of age (prediabetic stage) among the three groups (Fig. 2). These findings indicate that cPLA2α deficiency accelerates the development of insulin, not in the prediabetic stage, but in the diabetic stage, leading to the acceleration of diabetes. In other words, cPLA2α is likely to play a protective role in the acceleration of insulin in the diabetic stage.

In our previous reports, PGE2 production was significantly suppressed in peritoneal macrophages of cPLA2α−/− mice [7]. As the peritoneal macrophages are known to be involved in the development of diabetes in NOD mice [9–11], we investigated the production of PGE2 by the peritoneal macrophages of cPLA2α+/+, cPLA2α+−/−, and cPLA2α−/− NOD mice at 18 weeks of age. When peritoneal macrophages were stimulated with a Ca^{2+} ionophore (A23187) for 30 min or LPS for 8 h, PGE2 production was markedly lower in the cPLA2α−/− NOD mice than in the cPLA2α+/+ and cPLA2α+−/− NOD mice (Fig. 3A and B).

To evaluate whether cPLA2α deficiency affects the cytokine profile in APCs, we investigated the production of TNF-α and IL-12, which are considered to be involved in the development of diabetes in NOD mice, by peritoneal macrophages of cPLA2α+/+, cPLA2α+−/−, and cPLA2α−/− NOD mice at 18 weeks of age (Fig. 4). When peritoneal macrophages were stimulated with LPS for 8 h, TNF-α production was significantly higher in the cPLA2α−/− NOD mice than in the cPLA2α+/+ and cPLA2α+−/− NOD mice. This result is consistent with the report that TNF-α production from murine macrophages was inhibited by PGE2 [12]. On the other hand, IL-12 production levels from peritoneal macrophages could not be compared among the three groups (cPLA2α+/+, cPLA2α+−/−, and cPLA2α−/− NOD mice) because all of the data were below detection limit in our ELISA system.

Accumulating evidences proved that TNF-α has a potent effect on the development of diabetes in NOD mice. It has been reported that TNF-α is produced in macrophages or dendritic cells infiltrating in insulitis lesions [13]. Systemic administration of TNF-α to neonatal NOD mice accelerated diabetes...
A23187 was performed at 18 weeks of age, and the PGE2 production level was examined using ELISA. *P < 0.05 (by Mann–Whitney U test). Data are shown as the means ± SE.


In the immune system, prostanoids are produced mainly by APCs (macrophages and dendritic cells) and play an important role in regulating T-cell development and function [6]. In particular, PGE2 inhibits Th1 cell development from naive T cells [19–21]. Moreover, PGE2 suppresses IL-2 and IFN-γ production by Th1 cells, but not IL-4 and IL-5 production by Th2 cells [22,23]. Also, a recent study demonstrated that indomethacin- or EP4 (one of PGE2 receptor subtypes) antagonist-treated macrophages augmented their IL-12 production via an inhibition of PGE2 action and enhanced T cell-derived IFN-γ production after LPS-stimulation [24]. Therefore, the accelerated Th1-type immune responses evoked by inhibition of PGE2 production might contribute to the development of overt diabetes, although we could not observe the significant change in the cytokine profiles in our cPLA2α−/− NOD mice, at least for the pancreas and pancreatic lymph nodes (data not shown). In order to clarify the association between PGE2 deficiency and Th1-type immune responses, further studies using PGE2 deficient NOD mice are required in the future.

In conclusion, we demonstrated here that a cPLA2α deficiency resulted in decreased PGE2 production by peritoneal macrophages, leading to the increase in TNF-α production by APCs, contributing to the acceleration of diabetes. These findings suggest that cPLA2α is likely to play a protective role in the pathogenesis of autoimmune diabetes.

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References


