Amylase Gene Is Expressed in Pancreatic Beta-Cell Line and Increased by Th1 Cytokines

Sayaka Arai, Hiroki Shimura, Soichi Takizawa, Masashi Takahashi, Miho Aoyagi, Shoichiro Tanaka, Toyoshi Endo, Tetsuro Kobayashi

The Third Department of Internal Medicine, Interdisciplinary Graduate School of Medicine and Engineering, The University of Yamanashi, Japan

Abstract

We found novel auto antibodies against amylase (AMY) with high frequency in the sera from the patients with diabetes caused by autoimmune pancreatitis (DAIP) and the patients with pancreatic type T1 diabetes mellitus (T1D). AMY auto antibodies are also found in patients with type II diabetes mellitus (T2D) and type II diabetes mellitus with pancreatitis (T2DM). AMY auto antibodies have been thought to be expressed only in the exocrine pancreatic tissues, but not in the endocrine tissues. We hypothesized that the presence of AMY auto antibodies in the sera from patients with T1D and DAIP could be caused by the presence of AMY auto antibodies in the endocrine tissue. We investigated the expression of AMY in the endocrine tissue of MIN6 cells, a pancreatic beta-cell line, and found that AMY is expressed in the endocrine tissue. The expression of AMY was increased by Th1 cytokines. We also found that the expression of AMY in the endocrine tissue was increased in MIN6 cells co-cultured with human T cells. These results suggest that the presence of AMY auto antibodies in the sera from patients with T1D and DAIP could be caused by the presence of AMY auto antibodies in the endocrine tissue.

Hypothesis & Aim

I. AMY mRNA levels

Result

I. AMY mRNA levels

Fig. 1 Effect of Th1 cytokines to AMY mRNA expression levels in MIN6 cells

- Basal AMY expression was identified in MIN6 cells, but there was no AMY expression detected in the addition medium.
- Co-addition of IFN-γ + TNF-α synergistically increased AMY gene expression, but not AMY in MIN6 cells.

Fig. 2 Time Course Study

- MIN6 cells were fixed by 4% Parahormaldehyde on chamber slide glasses and stained by anti-AMY polyclonal antibody.
- We made double-staining of human pancreatic tissue sections and stained with the same antibody.

Fig. 3 Dose dependent study of cytokines expression levels in MIN6 cells

- AMY1 mRNA levels were analyzed by real-time PCR. AMY1 gene expression was especially increased by 48h culture.

Fig. 4 Immunostaining (MIN6 cells)

- A: Anti-AMY antibody (Ab) (polyclonal), No cytokine, hematoxylin (+)
- B: Anti-AMY Ab (polyclonal), IFN-γ + TNF-α, hematoxylin (+)
- C: Anti-AMY Ab (polyclonal), IFN-α, hematoxylin (+)
- D: Real-time PCR. MIN6 cells cultured with IFN-γ + TNF-α showed higher expression of AMY1 gene.

Fig. 5 Immunostaining (human pancreas)

- A: Anti-AMY monoclonal Ab. In this case, we did not confirm any staining parts.
- B: Anti-AMY polyclonal Ab. In this case, we confirmed the staining parts in the tissue.

Summary & Conclusions

1. In MIN6 cells AMY (oligocell type) gene was detected, but AMY2 (pancreatic type) gene was not. Ratio of the basal expression levels was 1:1 (AMY1: AMY2).
2. When MIN6 cells were cultured with IFN-γ + TNF-α, AMY1 and AMY2 were markedly increased.
3. Co-addition of IFN-γ + TNF-α increased the expression of AMY1 gene, but it was less in MIN6 cells.
4. MIN6 cells with no cytokines showed weak staining parts in the immunostaining.
5. In the human pancreatic tissue slice, we determined some staining parts in the immunostaining of AMY1 polyclonal antibody.
6. It was suggested that the presence of AMY in the endocrine tissue of MIN6 cells might be related with the cell damage in diabetes associated with autoimmune pancreatitis and pancreatic type T1 diabetes.