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SUMMARY OF DOCTORAL THESIS

Name: Md. Abdul Mazid

Title: Development of immunological assays specific for prostaglandins D_2 and J_2 series to study the regulation of their biosynthesis and the role in adipocytes

プロスタグランジンD_2とJ_2シリーズに対する特異的免疫測定法の開発と脂肪細胞でのそれらの生合成調節と役割に関する研究

Here, we attempted to develop enzyme-linked immunosorbent assays specific for prostaglandins (PGs) D_2 and J_2 series to study the regulation of their biosynthesis and the role in cultured adipocytes.

For the determination of PGD_2 produced by cultured cells in response to external stimuli, immunological methods would be convenient and useful. However, PGD_2 is unstable under the physiological conditions, so that it has been difficult to get a specific antibody for the parent PGD_2. In an attempt to get a specific antibody for PGD_2, we tried to prepare monoclonal antibodies for 11-deoxy-11-methylene-PGD_2, a novel, chemically stable, isosteric analogue of PGD_2. We successfully cloned a hybridoma cell line secreting a monoclonal antibody reacting specifically with the parent PGD_2. To develop the enzyme-linked immunosorbent assay (ELISA) for PGD_2, the immobilized antigen using the stable PGD_2 derivative was immunoreacted in a competitive manner with the monoclonal antibody in presence of free PGD_2. The optimization of the assay provided a sensitive calibration curve for PGD_2 from 0.32 pg to 0.18 ng with a value of 7.6 pg at 50% displacement. PGD_2 was almost stable during the ELISA condition. The developed assay method was useful for applying to the direct determination of PGD_2 in the culture medium of mouse 3T3-L1 adipocytes. The incubation of PGD_2 in the maturation medium of adipocytes at 37 ^{0}C caused the chemical conversion into PGJ_2 derivatives. The conversion became more evident after 6 h of the incubation. These findings indicate the importance of considering the optimal time for collecting the samples to be determined for PGD_2 before the conversion starts to occur. In this study, monoclonal antibodies were successfully prepared for PGD_2 using a protein conjugate of 11-deoxy-11-methylene-PGD_2 as an immunogen. The use of this isosteric analogue of PGD_2 is more advantageous because this compound is chemically more stable than parent PGD_2, allowing the preparation of more

specific antibodies for PGD_2. Moreover, our developed ELISA was applicable to the determination of the amount of PGD_2 in the cultured medium directly and accurately without extracting prostanoids as long as the samples are collected within several hours. Taken together, the current novel ELISA method is useful, reliable, and convenient for the quantification of PGD_2 formed by cultured cells in response to external stimuli.

Adipocytes play a crucial role in the storage of fats and mobilization of free fatty acids, and furthermore serve as endocrine cells. Furthermore, the changes in the quality of adipocytes are responsible for the generation of insulin resistance and other life-style diseases. Certain nuclear receptors are involved in the control of a series of events leading to adipogenesis as well as the increased size of adipocytes in obesity. Of these, peroxisome proliferator-activated receptor (PPAR) γ, a member of the nuclear hormone superfamily, is a master regulator of the differentiation of adipocytes. Since this receptor is a ligand-activated transcription factor, the endogenous ligands should be provided for the activation of PPARγ in adipose tissues. Originally, PPAR-related receptors were found to be activated by antidiabetic drugs. Later, certain fatty acids and some eicosanoids including PGs of J_2 series were found to activate PPARs as natural ligands. In adipose tissue in vivo, true endogenous ligands for PPARγ have not been identified with certainty because there are a diverse group of lipids and related compounds as candidate molecules. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) has been identified as a natural ligand for PPARγ to promote adipogenesis. However, it remains elusive about the ability of PPARγ-expressing adipocytes to produce PGJ_2 series and the role in the life cycle of adipocytes. Here, we developed an enzyme-linked immunosorbent assay specific for 15d-PGJ_2. The analysis using this method revealed the increase in the endogenous synthesis of immunoreactive 15d-PGJ_2 in cultured adipocytes during the maturation phase. Further studies using cyclooxygenase (COX) inhibitors clarified the contribution of endogeous 15d-PGJ_2 produced by mature adipocytes to up-regulation of fat storage in an autocrine manner. The present study also confirmed that the effect of 15d-PGJ_2 was linked with the terminal differentiation during the maturation phase based on our data. COX inhibitors such as indomethacin and aspirin were effective in inhibiting the expression of adipocyte-specific genes such as adiponectin, and leptin. On the other hand, exogenous 15d-PGJ_2 up-regulated the gene expression of those adipocyte markers in the presence of aspirin. These findings support the idea that endogenous 15d-PGJ_2 was formed in PPARγ-expressing adipocytes and contributed to the up-regulation of adipogenesis in an autocrine manner during the maturation phase. Moreover, it is also conceivable that Δ^{12}-PGJ_2 would also contribute to the increased adipogenesis. These findings provide useful information for the further studies on the regulation of the biosynthesis of endogenous PGJ_2 series and the roles in adipose tissues in vivo.