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Hematopoietic prostaglandin D_2 synthase in the chicken Harderian gland

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Abstract

The Harderian gland (HG), a sero-mucous secreting organ in the eye orbit, has long been recognized as immunologically important in chickens. During experimentation to characterize immune components of the gland, proteomics analysis revealed the presence of hematopoietic prostaglandin D synthase (H-PGDS). Extraction of total RNA followed by RT-PCR produced cDNA of 597 base pairs. DNA sequencing revealed nucleic acid and predicted amino acid sequences that were 99% aligned with the one published sequence for chicken H-PGDS of the spleen. Alignment with murine, rat, and human H-PGDS were 69, 69, and 66%, respectively. Ocular vaccination of chickens with a Newcastle Disease/Infectious Bronchitis vaccine (Mass.-Ark. Strain) induced an increase in H-PGDS expression determined by real-time PCR. Furthermore, immunohistochemistry of frozen HG sections showed positive stained cells for both H-PGDS and mast cell tryptase in the sub-epithelial cell layers of the HG ducts. Based on the potent vasoactive role of PGD_2, it appears that the chicken HG is a site of active mucosal immunity partially mediated by PGD_2 synthesized by H-PGDS in the gland.

Keywords: Prostaglandin; PGD_2; H-PGD synthase; Class-Sigma GST; Harderian gland

1. Introduction

Prostaglandin D_2 (PGD_2) is recognized as a potent lipid mediator produced in response to allergens activating mast cells through IgE binding (Goldsby et al., 2003). The effects of PGD_2 on allergic reactive symptoms of the eye (redness, mucous discharge, and eosinophil chemotaxis) were observed over 20 years ago (Abelson et al., 1983), and common allergens that induce reactions of the conjunctiva are able to increase PGD_2 release within a relatively short period of exposure (Proud et al., 1990; Aichane et al., 1993). Furthermore, nasal discharge linked to dust mite sensitivities contained high levels of PGD_2 (Horak et al., 1998). It is apparent that PGD_2 is an important mediator of eye-related allergic reactions.

PGD_2 is the end-product of the cyclooxygenase pathway in mast cells (Galli and Lantz, 1998) leading

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to its release from the cells for contribution to inflammatory responses and chemoattraction of cells expressing CRTH2 (Hirai et al., 2001) and DP (Monneret et al., 2001) receptors. The enzyme in immune cells responsible for the conversion of PGH_2 to PGD_2 is hematopoietic prostaglandin D synthase (H-PGDS). PGDS is known as either lipocalin-type (L-PGDS, brain-type or glutathione-independent) or hematopoietic (H-PGDS, spleen-type, or glutathione-requiring) depending on the tissue source of the enzyme and its binding of glutathione. H-PGDS is a member of the class-Sigma glutathione S-transferases (GST).

L-PGDS is typically found in the central nervous system tissues, male genital organs, and the heart (Urade and Hayaishi, 2000). It synthesizes the production of PGD_2 for sleep regulation and nociception. H-PGDS, on the other hand, is found in spleen, thymus, liver, kidney, small intestine, colon, lung, pancreas, ovary, and bone marrow (Ujihara et al., 1988; Thomson et al., 1998). Its primary immunological sources are macrophages (i.e., histiocytes and Kupffer cells) and dendritic cells (Urade et al., 1989), mast cells (Urade et al., 1990), and T_H2 cells (Tanaka et al., 2000). Due to its tissue distribution and role in immune cell-associated reactions, it is referred to as H-PGDS. In addition to glutathione binding, it requires divalent cation-mediated activation by Ca^{2+} and Mg^{2+} (Inoue et al., 2003).

H-PGDS is a 199 amino acid protein with a predicted molecular mass of 22 kDa. Thomson et al. (1998) designed a set of primers to produce cDNA from splenic RNA of chickens. The open reading frame of the chicken cDNA is 597 bp with no signal sequence, thus resulting in a cytosolic protein which also lacks any glycosylation sites. Although originally cloned from the spleen, chicken H-PGDS is highly expressed in the liver, kidney, and intestine, while only scarcely evident in the spleen (Thomson et al., 1998).

The chicken HG, a sero-mucous secretory organ associated with the nictitating membrane of the eye, has been extensively studied as a site of immunological reactivity (Scott et al., 1993). It is a source of IgM, IgG, and IgA, with the latter important in mucosal immunity of the upper respiratory and gastrointestinal tracts (Albini et al., 1974). Understanding the significance of a discreet organ like the HG in the eye of chickens provides an excellent model for examining the nature of allergic reactions of the eye, which would relate to humans and biomedical organisms (e.g., mice) that are otherwise not convenient to use for allergic reactivity studies.

Fractions of HG proteins collected by chromatofocusing that influenced the immunological activity of bursa of Fabricius cells co-cultured with phorbol dibutyrate (Noblet, 2002) were separated by electrophoresis and processed for proteomics analysis. Evidence for the presence of H-PGDS emerged from this analysis, which led us to utilize molecular biology to confirm H-PGDS in the HG. The objectives of this study were to identify H-PGDS through cDNA sequencing and to determine its expression through real-time PCR following ocular vaccination with a commercial Newcastle Disease/Infectious Bronchitis vaccine. Here, we sequence H-PGDS in the chicken HG and compare its predicted protein sequence to those of murine, rat, and human H-PDGS. Also, we report increased expression of mRNA for H-PGDS induced by ocular vaccination and the presence of H-PGDS and tryptase positive cells in the HG. Our work suggests that the chicken HG expresses H-PGDS for the production of PGD_2, which influences immunological activity in the HG following ocular exposure to antigens.

2. Materials and methods

2.1. Harderian gland collection and preparation

Chickens used for Harderian gland (HG) collection were Single Comb White Leghorns (10–14 weeks of age). For production of supernatant, HG were removed from the eye orbit, trimmed of excess tissue, and placed in 1 mL of cold PBS (Sigma Chemical Co., St. Louis) per gland. The glands were then homogenized using a Tissue Tearer (Biospec Products Inc., Batesville, OK) and centrifuged for 30 min at $10{,}415 \times g$ at 4 °C. The homogenate supernatant was then dialyzed overnight in PBS at 4 °C with a 7 kDa molecular weight cutoff. The dialyzed sample was filter-sterilized using a 0.2 μm Sterile Acrodisc filter (Gelman Sciences, Ann Arbor, MI) and stored at 4 °C.

For RNA isolation, HG were removed as described above using baked instruments and placed in 1 mL of