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Expression of different classes of immunoglobulin in intraepithelial plasma cells of the Harderian gland of domestic ducks *Anas platyrhynchos*

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Abstract

The Harderian gland of chickens contains numerous plasma cells and is considered as a peripheral lymphoid organ. Data about this gland in other avian species are scarce or inexistent. Considering that ducks show some unique characteristics regarding the immune system, which are important in evolutionary context, and that unusual location of plasma cells into the epithelium was recently described in primitive avian species, here we investigated the occurrence and characterized intraepithelial plasma cells in the Harderian gland of ducks, according to the immunoglobulin produced. Numerous intraepithelial plasma cells were found confined to the Harderian gland ducts. Plasma cells were also found in the ducts lamina propria. IgM-positive cells were the most abundant into the epithelium. In contrast, IgY- or IgA-positive cells were predominant in the lamina propria. The constancy of intraepithelial plasma cells in all specimens examined indicates that they may be essential mediator for an effective immunesurvaillance of the ocular mucosa.

Keywords: Harderian gland; Intraepithelial plasma cells; Immunoglobulin; Mucosal immunity; Ducks

1. Introduction

The Harderian gland is located in the eye orbit and plays a role in the production of fluid that lubricates the nictitating membrane and protects the cornea of most terrestrial vertebrates (Shirama et al., 1996). Birds differ from other vertebrates due to the presence

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of unusually large number of plasma cells in the interstitium of the Harderian gland (Bang and Bang, 1968; Wight et al., 1971; Aitken and Survashe, 1977; Mansikka et al., 1989; Shirama et al., 1996; Altunay and Kozlu, 2004), which produce and secrete different classes of immunoglobulin, i.e. IgY, IgA and IgM (Albini et al., 1974; Oláh et al., 1992; Scott et al., 1993). The Harderian gland is also a site of activation and terminal differentiation of B-cells, as well as plasma cell proliferation and immunoglobulin class switch, as seen in chickens (Gallego and Glick, 1988; Mansikka et al., 1989; Savage et al., 1992; Scott et al., 1993; Tsuji et al., 1993; Oláh et al., 1996; Koskela et al., 2003). Based on these distinct features, the chicken's Harderian gland has been considered as a peripheral lymphoid organ, participating in local immune responses (Shirama et al., 1996). In spite of the evidences suggesting the important role of the Harderian gland in the chicken immune protection, data about the gland in other avian species are scarce or even inexistent.

Plasma cells are typically found in the connective tissue and lymphoid tissue, but are rarely seen within the epithelium. However, a large number of intraepithelial plasma cells was recently described in the copulatory organ of some tinamous birds (Oliveira et al., 2003a) and domestic ducks (Oliveira et al., 2003b). Similar occurrence of plasma cells into the epithelium of the copulatory organ (von Rautenfeld et al., 1976) and Harderian gland of chickens (Bang and Bang, 1968; Oláh et al., 1992, 1996) has also been reported, suggesting that this intraepithelial location of plasma cells are more common in birds than first predicted and may play an important role in the avian mucosal immune system.

While sharing with chickens some common features, ducks also show some unique characteristics regarding the immune system, such as (1) the occurrence of lymph nodes, (2) the occurrence of lymphoid annular bands in the gastrointestinal tract (Ellsworth and Ellsworth, 1981), (3) the ineffective immune response, considering the predominance of antibodies deficient in some biological effector mechanisms (Higgins and Warr, 1993; Warr et al., 1995), and (4) the delayed IgA-dependent mucosal immune response development (Magor et al., 1998). In addition, ducks are among the most primitive extant birds (Higgins and Chung, 1986); therefore they are a species of interest for elucidating phylogenetic immunological relations. Although the gross structure, histology, ultrastructure and some histochemistry of the Harderian gland of ducks have been reported (Fourman and Ballantyne, 1967; Brobby, 1972; Wight and Mackenzie, 1974; Aitken and Survashe, 1977; Maxwell and Burns, 1979), studies dealing with the local plasma cell population, and the importance of the gland in the immunologic mechanisms are still missing.

The lack of information about the immunological properties of the duck Harderian gland, besides the intriguing findings about the unusual location of plasma cells confined to some restricted areas of avian mucosa, prompted us to investigate the occurrence and characterize the intraepithelial and interstitial plasma cells of the Harderian gland of ducks, according to the immunoglobulin type produced. The information obtained may be relevant for the understanding of the immune system of domestic birds.

2. Materials and methods

2.1. Animals

The investigation was performed on Harderian glands of 22 adult domestic ducks *Anas platyrhynchos*, obtained from commercial sources and housed in the Federal University of Minas Gerais facilities. The animals were maintained in natural conditions of light, humidity and temperature, and were allowed free access to water and food (Socil-III/Guyomarc'H; Belo Horizonte, Brazil). The principles of research involving animals followed those expressed in the ''Princípios éticos para o uso de animais em experimentação'', advocated by the ''Comité de ética em pesquisa'', published by the Federal University of Minas Gerais (UFMG) (http://www.ufmg.br/coep/cetea.html).

2.2. Histology

The ducks were weighted, anesthetized (i.p. sodium pentobarbital 50 mg/kg body weight), and perfused intracardially with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 or with 10% neutral buffer formalin. After fixation, the Harderian glands