NOTE \ Anatomy

Distribution of Immunoglobulin Isotypes and Subisotypes in Equine Guttural Pouch (Auditory Tube Diverticulum)

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(Received 19 January 2000/Accepted 19 May 2000)

ABSTRACT. To clarify the functions of the equine guttural pouch, the distribution of various immunoglobulin isotypes and subisotypes in the guttural pouch mucosa were examined in healthy horses. IgGa was present in the mucosa of guttural pouch, mucosal lymph nodes and submucosal lymph nodules. IgM was scattered in the mucosal lymph nodules and in the germinal centers of the submucosal lymph nodules. IgGc was recognized only in the submucosal lymph nodules. These immunoglobulin isotypes and subisotypes were found in lymphocytes and plasma cells. On the other hand, IgA was detected in glandular epithelial cells and the surface layer of the mucosal epithelium, as well as in free cells. This finding suggests that IgA is secreted through the glandular epithelium. Based on the above findings, we conclude that the guttural pouch has phylactic ability.

KEY WORDS: equine, guttural pouch, immunoglobulin isotype.

The auditory tube diverticulum in equines and tapis is called as guttural pouch based on its distended structure with voluminous diverticular compartments, and is an organ characteristic of solipeds. It is in contact with the caudopharyngeal cavity [1–3, 8, 11]. The pouch mucosa has cilia, a structure for removing foreign substances, and intensively developed mucosa-associated lymphatic tissues beneath the epithelium, suggesting that this organ has high phylactic ability [4, 5, 12]. However, only a few reports are available concerning the mucosal immunity of equines. To contribute to the study on the phylactic system of the guttural pouch, we conducted an immunohistochemical examination using monoclonal antibodies to investigate the distribution of immunoglobulin isotypes and subisotypes in equine guttural pouch mucosa.

Two female thoroughbreds (nine and eleven years old) were used in this study. The animals were anesthetized with an intravenous injection of a mixture of 2 g sodium thiopental (Ravonal\textsuperscript{a}; Tanabe Seiyaku Co., Osaka, Japan) and 100 mg suxamethonium chloride (Succine\textsuperscript{a}; Kyorin Pharmaceutical Co., Tokyo, Japan), and subsequently sacrificed by severing of the carotid arteries. These animals did not display any clinical abnormalities at the time of pathological examination.

For immunohistochemical analysis, three samples each were taken from three regions of the posterior mucosa in the medial components of the guttural pouch, where mucosa-associated lymphatic tissues were well developed (Fig. 1a). These three regions are indicated on the silicon mold of the entire guttural pouch (Fig. 1b). The mold was obtained in our previous study [8]. After being fixed with periodate-lysine-paraformaldehyde (PLP) [9, 10] cooled to 4°C, the sampled tissues were rinsed in phosphate buffer saline (PBS), embedded in paraffin according to the conventional method. They were then cut into 3 mm-thick sections and subjected to the enzyme-labeled antibody method [10] using streptavidin-biotin complex (S-ABC, VECTASTAIN\textsuperscript{b}, Funakoshi, Co., Tokyo, Japan). As primary antibodies, monoclonal antibodies against equine IgGab, IgGa, IgGb, IgGc, IgG (T), IgM, and IgA, which were established by Sugiura et al. [13], were used in dilution of 1:2,000 with PBS. Monoclonal antibody against IgGab reacts to both IgGa and IgGb. The specificity of monoclonal antibodies was confirmed at the second workshop on the horse leukocyte antigen, California, U.S.A., 1996 [7, 13]. Before immuno-histochemical staining, we treated the samples with 0.1% Actinase (pronase) (Kaken Pharmaceutical Co., Tokyo, Japan) at 37°C for 5 min after deparaffinization.

IgGab and IgGa positive cells were found in lymphocytes distributed in the mucosal lymph nodules and marginal region of germinal centers of the submucosal lymph nodes, as well as in plasma cells scattered in the mucosa and in medulla of the submucosal lymph nodes (Fig. 2a). No IgGb-positive cells were identified. Only plasma cells diffused in the submucosal lymph nodules were IgGc-positive. No IgG (T)-positive cells were identified. IgM-positive cells were distributed in almost the same region with IgGab-positive cells. The former was diffused evenly in the mucosal lymph nodules and in the germinal center of the submucosal lymph nodes (Fig. 2b). IgA-positive cells were recognized in the lamina propria immediately beneath the epithelium, especially among free cells distributed in the margin of glandular tissues. Some glandular epithelial cells

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were slightly positive (Fig. 2c). Positive substances were sporadically seen in the glandular cavities and in the surface layer of the epithelium around glandular openings (Fig. 2d).

 Since the guttural pouch opens to the nasopharynx through the pharyngeal ostium of the auditory tube, it is easily invaded by foreign substances. To eliminate such substances, the guttural pouch is equipped with cilia on its mucosal epithelium. The organ also has well-developed lymph nodules in the mucosa. These lymph nodules may react to antigens of foreign substances. These findings indicate that the guttural pouch is an organ having not-negligible immune system.

 Although equine immunoglobulin isotypes and subisotypes have been well described [6], their functions are not fully understood. The present results demonstrated the presence of IgGa, IgGe, IgM, and IgA in the guttural pouch mucosa of healthy horses. IgGab-positive cells seems to produce only IgGa because IgGb-positive cell was not demonstrated. These immunoglobulin isotypes and subisotypes were all seen in free cells, such as lymphocytes and plasma cells. On the other hand, IgA was found in glandular epithelial cells and on the surface of the mucosal epithelium as well as in free cells, suggesting that IgA may be secreted through the epithelial cells of glandular ducts. These findings led us to conclude that the guttural pouch has phylactic ability.

 ACKNOWLEDGMENTS. We would like to express our deep appreciation for the cooperation of the staff at the Japan Racing Association, the Equine Research Institute, the Clinical Sciences and Pathobiology Division, and the Epizootic Research Station, Microbiology Division.

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Fig. 2. Distribution of immunoglobulin isotypes and subisotypes in the guttural pouch mucosa. × 340. (a): region ③. Distribution of IgGα and IgGα in the guttural pouch mucosa. IgGα and IgGα positive cells are found in lymphocytes distributed in the marginal region of the mucosal lymph nodule and germinal center of the submucosal lymph nodules. (b): region ③. Distribution of IgM in the guttural pouch mucosa. IgM-positive cells are evenly distributed in the lymph nodule and germinal center of the submucosal lymph. (c): region ③ and (d): region ②. Distribution of IgA in the guttural pouch mucosa. IgA-positive cells are recognized in the lamina propria immediately beneath the epithelium, especially among free cells distributed in the margin of glandular tissues (large arrows). Some glandular epithelial cells are slightly positive (small arrows). IgA-positive substances are seen in the glandular openings (arrowheads).