

# The immunohistochemical localization of secretory IgA in the submandibular gland of the Mongolian gerbil

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## Abstract

**Introduction:** Immunoglobulin A (IgA) plays an important role in local mucosal immunity. IgA-containing cells mainly occur in the duct system and the lumen of the glandular acini in the human submandibular gland.

**Material and methods:** Immunohistochemistry technique was employed to survey the distribution of IgA in the submandibular gland of the Mongolian gerbil. Forty Mongolian gerbils consisting of 5 homozygous males and 5 homozygous females aged 21d, 90d, 360d and 720d were selected.

**Results:** The gerbil submandibular gland can be discriminated into a secretory portion and a duct portion. The former mainly included serous acini and the latter contained intercalated ducts, striated ducts, granular convoluted tubules and interlobular ducts. IgA can be regularly visualized by 80°C heat isotope antibody retrieval (HIAR) after neutral formaldehyde fixation. The 1:100 HRP-conjugated goat anti-rat IgA is an effective antibody for evaluation of the IgA distribution in the gerbil. The results also demonstrated that the incubation time and temperature of primary antibody also influenced the staining results. IgA-positive cells were regularly presented in serous acini, intercalated ducts, striated ducts, granular convoluted ducts and interlobular ducts. They were also visualized in the connective tissues among the acini and ducts. The staining pattern appeared uneven among different secretory regions and duct regions. There were no regular changes according to the age. The positive reaction product was localized in the cytoplasm, while the nucleus was negative.

**Conclusions:** IgA from local plasma cells or contributed from blood were transported into epithelial cells of acini and ducts by way of connective tissues underneath them.

**Key words:** immunoglobulin A, submandibular gland, immunohistochemistry, Mongolian gerbil.

## Introduction

Immunoglobulin A (IgA), together with polymeric IgA, is the most important and abundant immunoglobulin in mucosal secretions, e.g. saliva, tears [1]. It plays an important role as a local agent in mucosal immunity, especially by preventing bacterial adhesion, inhibiting viral attachment, diminishing parasitic infestation and blocking toxin-induced damage to local mucosal tissues, while preventing it interfering with gastrointestinal function [2]. In previous studies, it was presented that immunoglobulin A-containing cells are widely distributed in the mucosal tissues along the gastrointestinal tract [3]. Studies from many laboratories have suggested

that the predominance of IgA-producing plasma cells in the parenchyma of salivary glands [4, 5] revealed that the IgA-positive reactions mainly occurred in the intercellular connective tissue and the lumen of glandular acini and ductules in human submandibular and parotid salivary glands. The origin of IgA plasma cells of the salivary gland has not been identified yet. A possible induction site for salivary IgA is the nasal lymphoid tissue [6, 7]. Situ et al. also presented that secretory component was mainly localized in the glandular ductules and serous acini [8]. So far there are only a few documents about the distribution of secretory IgA in the salivary gland. To some extent, these findings are still incompatible with each other. However, although the Mongolian gerbil has been widely used in many aspects of biomedical research, the biological properties of this laboratory animal are not completely clear. In this study, the distribution of IgA in the submandibular glands of the Mongolian gerbil was evaluated.

## Material and methods

### Animals and treatments

The gerbil was captured from Inner Mongolia in 1978, and has been maintained as a separate culture at the Zhejiang Laboratory Animal Centre in China since then. Forty Mongolian gerbils, consisting of 5 homozygous males aged 21d, 90d, 360d and 720d, and 5 homozygous females aged 21d, 90d, 360d and 720d, were selected. All animals were kept in animal cabinets and placed in conventional rooms until use. In the sampling procedure, animals were anaesthetized with carbon dioxide. Then the left submandibular gland samples were collected and fixed in 10% neutralized formalin solution for 18-24 hours.

### Histological examination

The submandibular samples of gerbils were fixed in 10% neutral buffer formalin for another 18 hours. The tissue samples were processed by routine methods to paraffin-embedded blocks. The tissues were serial sectioned at 6-8  $\mu\text{m}$ . The sections (8  $\mu\text{m}$ ) were stained with haematoxylin and eosin (HE) as normal. After that, the sections were observed by an Olympus microscope and photographed.

### Immunohistochemistry

HRP-conjugated goat anti-rat IgA antibody (Serotec, Oxford, UK), HRP-conjugated goat anti-mouse IgA (Zymed, California), and HRP-conjugated staphylococcal protein A were employed as the primary antibody. Tissue sections were dewaxed in xylene and rehydrated in a graded alcohol series. Antigen retrieval was performed by immersing sections in EDTA antigen retrieval solution and

heating at 80-98°C for 30 min in a water bath, and allowing them to cool naturally. The sections were then immersed in cool phosphate buffer with salt (PBS, 10 mM, pH 7.4) for 10 min, and rinsed in PBS. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in distilled water for 10 min at room temperature. The sections were then rinsed in PBS, and incubated for 30 min at room temperature with 10% normal goat serum. Subsequently, they were incubated in primary antibody (diluted 1:50 to 1:200 with distilled water) at 4°C overnight (for more than 12 hours or at room temperature for 2 hours). Substitution of PBS for the primary antibody served as a negative control. The sections were then rinsed in PBS three times. After that, the sections were incubated in peroxidase substrate solution (Lab vision, Fremont, UK) for 15 min at room temperature. The sections were rinsed in distilled water, and counterstained with Mayer's haematoxylin for 1 min [9]. Then the sections were dehydrated, cleared and mounted as normal, observed by Olympus microscope and photographed.

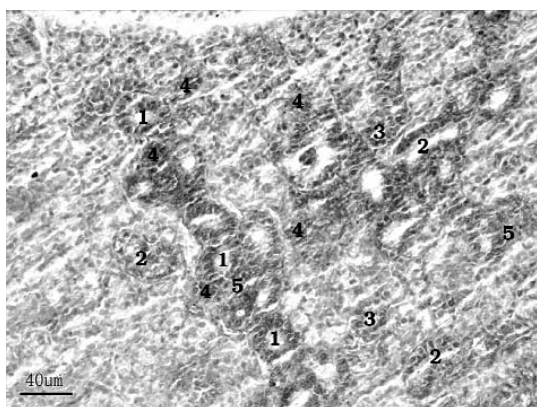
## Results

### Histology examination

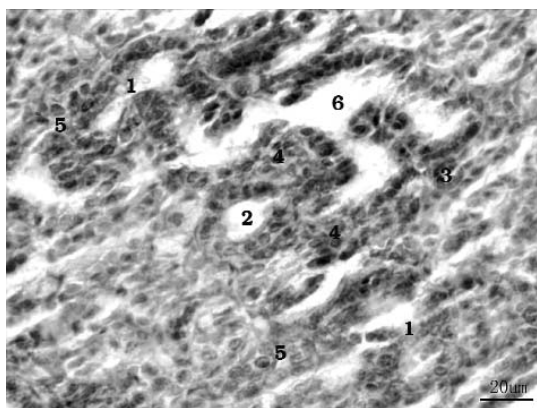
The submandibular gland of the gerbil was divided into many lobules by connective tissue. The parenchyma consisted of a secretory portion and a duct portion. The former mainly consisted of deep-stained serous acini. The light-stained mucous acini and mixed glandular acini only occupied a small amount. The drainage duct can be distinguished as the intercalated duct, striated duct, granular convoluted tubule and interlobular duct. The granular convoluted tubules were mainly composed of simple columnar epithelium, which presented acidophilic granules on its apical cytoplasm. There were one to several layers of myoepithelial cells around different acini and ducts.

### Influence of immunohistochemical procedure on IgA visualization

IgA was regularly visualized in submandibular gland samples fixed by formaldehyde. The IgA-positive reactions were localized more precisely and stained more intensively in 80°C heat isotope antibody retrieval (HIAR) than HIAR at other temperatures. The HRP-conjugated goat anti-rat IgA was the best agent for all three tested primary antibodies to capture IgA in tissue. The results revealed that the optimal work dilution of the HRP-conjugated goat anti-rat IgA antibody was 1:100. The results also demonstrated that the incubation time and incubation temperature also influenced the staining results. A high contrast staining pattern could be acquired by incubating with primary antibody at 4°C overnight or over 12 hours after 80°C HIAR.



**Figure 1.** The distribution of IgA-positive reaction in the submandibular gland of the Mongolian gerbil. The figure presents the distribution of IgA-positive reactions in the submandibular gland. The positive reaction was mainly distributed in the duct system and only a little was found in the serous acini: 1 – striated duct, 2 – glandular convoluted tubules, 3 – intercalated duct, 4 – serous acini, 5 – connective tissue among the glandular acini and glandular ducts. Visualized by DAB, 20x objective lens, 2040\*1536 pixels, electronically imaged, scale bar in the photo equalled 40 μm



**Figure 2.** The localization of IgA-positive cells of the submandibular gland in the Mongolian gerbil. The photo is presented to demonstrate the distribution of IgA-positive cells in the submandibular gland. The DAB stained cells were mainly localized in the following tissues: 1 – striated duct, 2 – glandular convoluted tubules, 3 – intercalated duct, 4 – serous acini, 5 – connective tissue among the glandular acini and glandular ducts, 6 – interlobular duct. Visualized by DAB, 40x objective lens, 2040\*1536 pixels, electronically imaged, scale bar in the photo equalled 20 μm

### Localization of IgA in submandibular gland

All negative control sections presented no DAB staining products in the connective tissues and the parenchyma. The cytoplasm of positive cells was stained brown-yellow to brown-black. The staining

results showed no regularly staining pattern associated with each age treatment. IgA-positive products were regularly presented in the epithelial cells of the serous acini, intercalated ducts, granular convoluted tubules, striated ducts and interlobular ducts of all tissue specimens in different age groups. The mucous acini were generally stained negative (Figure 1). Occasionally, extremely weak positive reactions were observed in mucous acini, particularly in the borders of the epithelial cell in mucous acini. A positive reaction was also diffusely distributed in the connective tissue stroma among the glandular acini and glandular ducts. A general intensification was observed in the vessel walls and basement membranes. The stained intensity of the central cytoplasm of the epithelial cells was relatively weaker than the border. In addition, an intensive staining rim of IgA was found, particularly in the striated ducts, granular convoluted tubules and some intercalated ducts (Figure 2).

### Discussion

IgA is the most important agent in the mucosal immune response. There are two molecular forms, namely monomeric IgA and polymeric IgA, which are composed of disulphide-linked monomers. A small glycoprotein called secretory component links the IgA molecules to the epithelial cells by a disulphide bond. Therefore, the distribution of IgA in the submandibular gland can reveal the local IgA transport patterns. It also presents the distribution patterns of secretory component in the epithelial cells of glandular acini and glandular ducts. It was reported that the IgA cells were localized in the stroma between the acini, and visualized in the cells adjacent to the striated ducts in the human submandibular gland. Meanwhile, the IgA was selectively presented along the lateral aspects of the epithelial cells as well as in the apical part of their cytoplasm [2, 4, 8, 10]. In the present study, the results revealed that the IgA was mainly localized in the epithelial cells of the serous acini, intercalated ducts, granular convoluted tubules, striated ducts and interlobular ducts, which is somewhat different from those findings. The present results also showed that the IgA was diffusely distributed in some serous acini, while other serous acini showed negative results, which suggested that the IgA molecules were distributed unevenly in the secretory portion of the submandibular gland. It is very likely that the serous acini were in different stages of development, while differently developed acini showed different affinity to IgA. Probably, the forming stage or immature serous acini presented as IgA negative, while the epithelial cells of mature acini could present partial to fully IgA positive patterns. The current research also demonstrated that the secretory part of the

whole glands showed weaker staining than the drainage parts. It was obvious that the IgA produced by plasma cells or serum-contributed was previously transported to the duct regions.

It is well known that the IgA was mainly synthesized by the plasma cells distributed in the mucosal membrane or connective tissue. By selective non-covalent affinity for the dimeric IgA, the plasma membrane associated secretory components mediated and determined the IgA uptake of serous epithelial cells [11, 12]. It is easy to understand that the IgA in these cells was also diffused into the lumen [13, 14]. In the present study, the IgA-positive reaction in the free edge and basal edge of epithelial cells was relatively intense, which indirectly supported the IgA transport pathway by which most of the IgA was transported across the epithelium into the lumen after binding to the poly-Ig receptor [15, 16]. It was also similar to those findings in humans [4]. The IgA was also localized in the connective tissues among glandular acini and ducts. It followed from this that the A-containing plasma cells were previously transported to the connective tissues and the IgA contributed by blood was also selectively localized to connective tissues under the epithelial cells. It must be pointed out that the distribution of the mediating substance (e.g. secretory component) to link IgA to epithelial cells determined the distribution of IgA in the whole gland. But it is still unclear whether there is only secretory component or there also exists another substance influencing the local distribution of IgA-producing plasma cells and the allocation of blood IgA. A possible way is that the IgA produced by plasma cells or contributed by blood was first selectively transported into the epithelial cells, which was adjusted by the mediating substances.

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