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Research paper

NALT (nasal cavity-associated lymphoid tissue) in the rabbit

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ABSTRACT

Due to its many advantages, interest in intranasal vaccination of domestic mammals and humans is currently increasing. Successful stimulation of the immune system by intranasal vaccines requires, however, the presence of lymphoid tissue in the nasal cavity. This nasal cavity-associated lymphoid tissue (NALT) has already been described in humans and many laboratory rodents, but data about rabbits are very scarce. For this purpose, histological sections of the nasal cavities of 10 female adult New Zealand White rabbits were examined for the presence of lymphoid tissue. Primary (I) and secondary (II) lymphoid follicles divided by interfollicular regions were mainly present at the bottom of the ventral nasal meatus and the nasopharyngeal meatus from 1 to 3.3 cm from the tip of the nose. In this region intraepithelial and lamina propria lymphocytes, and isolated lymphoid follicles (ILF's) were additionally seen at the dorsal and dorsolateral sides of the nasopharyngeal meatus and within the mucosae of the nasal conchae and the lateral nasal walls. Intraepithelial and lamina propria lymphocytes, and ILF's were, just like in humans, randomly distributed along the entire nasal mucosa. The rabbit NALT is more voluminous compared to rodents in which lymphoid tissue is only present at the bottom of the nasopharyngeal meatus. Since the relative volume of the rabbit nasal cavity is also similar to that of humans, the rabbit could be a valuable research model not only for animal but also for human intranasal vaccine development.

1. Introduction

The lymphoid tissue present in the nasal mucosa is named nose- (or nasal cavity) or nasopharynx-associated lymphoid tissue (NALT) which is part of the mucosa-associated lymphoid tissue (MALT) (Brandtzaeg and Pabst, 2004). Primary and secondary B cell follicles and interfollicular T cell regions act as inductive sites, and intraepithelial and lamina propria lymphocyte as effector sites for mucosal immunity (Brandtzaeg and Pabst, 2004). In rodents, NALT is of particular interest as they lack tonsils (Liang et al., 2001). These structures, officially named *lymphonoduli aggregati*, are well organized lymphoid tissues consisting of aggregated primary and secondary lymphoid follicles separated by interfollicular areas where mainly T cells accumulate. The covering epithelium is often infiltrated by lymphoid cells forming a reticular epithelium (Oláh et al., 2003; Anonymous, 2005). Consequently, NALT has already been described in many laboratory rodents, including the mouse, rat, hamster, guinea pig and chinchilla (Hameleers et al., 1989; Spit et al., 1989; Koornstra et al., 1991; Okada et al., 1995; Giannasca et al., 1997; Heritage et al., 1997; Wu et al., 1997; Asakura et al., 1998; Liang et al., 2001; Jurcisek et al., 2003; Kraal, 2004). In all these species it consists of bilaterally symmetric lymphoid tissue that is located in the ventrolateral mucosa of the nasopharyngeal meatus. Additionally, intraepithelial and lamina propria lymphocytes are present in the mouse (Asanuma et al., 1998; Liang et al., 2001; Hobson et al., 2003).

In man, well organised NALT is represented by the pharyngeal and tubal tonsils that are located in the nasopharynx (Koornstra et al., 1991; Kuper et al., 1992, 2003; Boyaka et al., 2000). Until recently, intraepithelial

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and lamina propria lymphocytes, and ILF's that are randomly distributed in the nasal cavity only received little attention since many authors considered it as less important compared to the nasopharyngeal tonsils. Moreover, investigating this lymphoid tissue is technically not evident in men (Debertin et al., 2003, 2006; Liebler-Tenorio and Pabst, 2006). Only Debertin et al. (2003) report the presence of aggregations of lymphoid cells overlaid by a follicle-associated epithelium (FAE), distributed along the nasal cavity of young children.

In contrast, NALT in the rabbit has never been investigated systematically. It has only been illustrated briefly in a study of the rabbit nasal cavities by Kelemen (1955), and in an experiment about polypeptide transcytosis in the rabbit nasal mucosa (Cremaschi et al., 1998). Neither of both studies mentions the exact location of the lymphoid tissue, nor do they describe the lymphoid structures in detail. Investigating the rabbit NALT is, however, impelled by the increasing interest in human, but also animal intranasal vaccination for which the rabbit could serve as a prospective animal model (Gizurarson, 1990; Kuper et al., 1992; Illum, 1996; Renauld-Mongénie et al., 1996; Levine and Dougan, 1998; Levine, 2003; Gizurarson et al., 2006). Our study therefore aims to systematically investigate the presence of NALT in the rabbit.

2. Materials and methods

2.1. Animals

In the present study 10 adult female New Zealand White rabbits weighing approximately 2 kg were used. The animals were euthanised for educational reasons by means of an injection of 1 ml T61® (Embutramide 200 mg, Mebenzoniumiodide 50 mg, Tetracaine hydrochloride 5 mg, Dimethylformamide et aqua dest. q.s. ad 1 ml, Intervet, Mechelen, Belgium) in the lateral ear vein. After death, they were decapitated in the atlanto-occipital joint. The nasal cavities were cross-sectioned in five 1 cm thick blocks using a band saw (Fig. 1).

2.2. Histological analysis

All blocks were fixed for 1 week in 3.5% buffered formaldehyde at room temperature. They were subsequently decalcified at room temperature by immersion in a solution consisting of 90 ml distilled water, 5 ml formic acid and 5 ml 3.5% buffered formaldehyde. The solution was replaced several times until no more precipitating calcium could be detected after adding 0.1 ml ammonium-oxalate to 5 ml of the solution which had first been neutralized by the addition of NaOH (Gooding and Stewart, 1932). Decalcification was completed after approximately 3 weeks. The blocks were then dehydrated in a tissue processor (STP420D, Prosan, Merelbeke, Belgium) and embedded in paraffin using an embedding station (EC 350-1, Prosan). From the paraffin blocks 8 μm thick sections were cut at an interval of 0.5 mm using a microtome (HM 360 microtome, Prosan). All sections were mounted on slides and automatically stained with haematoxylin and eosin (Linear Stainer II, Sakura Finetek Europe B.V., Zoeterwoude, The Netherlands). A motorised light microscope (Olympus BX 61, Olympus Belgium, Aartselaar, Belgium) linked to a digital camera (Olympus DP 50, Olympus Belgium) was used for histological examination.

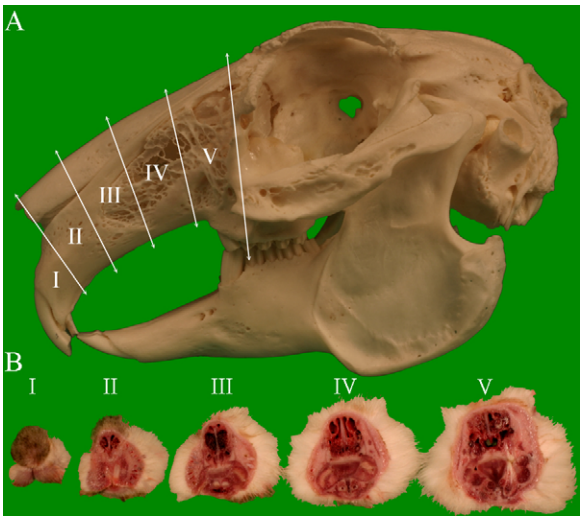

Fig. 1. (A) Rabbit skull showing the positions of the five 1 cm thick cross-sections (I–V) used for sampling tissue blocks for histological sectioning at an interval of 0.5 mm. (B) Rostral views of the five (I–V) fresh blocks.

3. Results

In the first block (0–1 cm from the tip of the nose) (Fig. 2) intraepithelial and lamina propria lymphocytes,

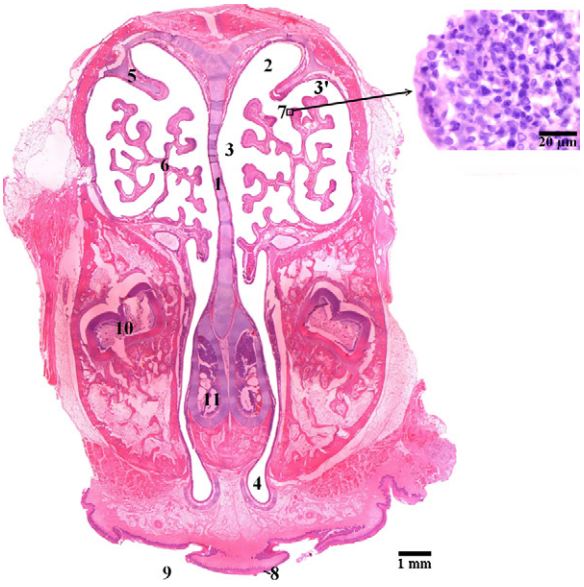

Fig. 2. Histological section of a decalcified rabbit nasal cavity at the level of block I: nasal septum (1), dorsal nasal meatus (2), common nasal meatus (3), middle nasal meatus (3′), ventral nasal meatus (4), dorsal nasal concha (5), ventral nasal concha (6), spiral lamellae containing intraepithelial and lamina propria lymphocytes, and small ILF's (7), hard palate (8), oral cavity (9), root of the upper incisor (10) and vomeronasal organ (11). Insert: lamina propria lymphocytes in a spiral lamella.