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Structural Analysis of the Mab213-Immunoreactive "Necklace Glomeruli" in the Rat Primary Olfactory System

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Abstract The aim of the present study was to clarify detailed distribution of the Mab 213-immunoreactive (Mab 213-I) olfactory receptor cells and glomeruli in the rat olfactory bulb, to elucidate the glomerular relationship with intensely acetylcholinesterase-reactive patchy regions, and to characterize the ultrastructural features of Mab 213- I glomeruli.

The great majority of Mab 213-I olfactory receptor cells were distributed over dorsal portions of the nasal epithelium at the caudal end of nasal cavity. Especially, such receptor cells were concentrated on the dorsal roof of epithelium adjacent to the nasal septum and near the caudal end of the turbinate 1. The Mab 213-I glomeruli were found to form a necklace pattern surrounding the caudal end of the olfactory bulb, one of them being located in the "modified glomerular complex" reported to be involved in rat suckling behavior. In addition, they were found to correspond to less reactive parts of intensely acetylcholinesterase reactive patchy regions which are next to the strongly reactive parts. The Mab 213-I glomeruli were clarified to have dark nodules in toluidine-blue-stained sections and characterized by the olfactory nerves rich in large dense-cored vesicles in immuno-electron microscopy. The present results strongly suggest that the Mab213- I "necklace glomeruli" may be the same subset of previously reported Shinoda's "necklace glomeruli".

Key words : olfactory bulb, olfactory receptor cells, dense-cored vesicles, acetylcholinesterase, suckling behavior

Introduction

The "necklace olfactory system" identified by the polyclonal antibody against human placental antigen X-P 2 (hPAX-P 2), is a distinct unit which has been thought to be involved in processing some olfactory information related to induction of rat suckling behavior[1]. The system is characterized by the presence of necklace-patterned terminal glomeruli and interglomerular fibers at the caudal end of the olfactory bulb[1]. The necklace glomeruli are intimately associated with intensely acetylcholinesterase-reactive (IAE) patchy regions which are composed of two distinct portions, strongly and less acetylcholinesterase reactive portions (IAE -S, IAE-L)[2]. They were found to correspond to the IAE-L which are located adjacent to the IAE-S[2]. The glomeruli can also be characterized by the presence of dark nodules in toluidine-blue-stained sections and presence

of olfactory nerves containing large dense -cored vesicles in electron microscopy[3]. Similar necklace-typed glomeruli have recently been reported to be present at the posterior margin of the rat olfactory bulb. Glomeruli containing cGMP-stimulated phosphodiesterase (PDE 2) were immunohistochemically demonstrated to be distributed in a similar necklace pattern[4]. The PDE 2 -immunoreactive glomeruli were also shown to be located adjacent to the IAE-S and speculated to correspond to the Shinoda's "necklace glomeruli". In addition, very recently, Ring et al.[5] also reported that one of the monoclonal antibodies against transforming growth factor-alpha (TGFα), which was designated as Mab 213 (clone 213-4.4), can immunohistochemically reveal another type of similar necklace glomeruli. Detailed structures of these glomeruli, however, remain to be clarified. The present study tried to clarify morphological features of the Mab 213-immunoreactive (Mab 213-I) primary olfactory system in more details, including the topographic organization, glomerular relationship with the IAE patchy regions and the ultrastructure of the Mab 213-I glomeruli.

Materials and Methods

Animals and tissue preparation

Twelves male wistar rats (postnatal one month) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused intra-cardially with 20-50ml of ice-cold saline followed by 0.1 M sodium phosphate buffer (pH 7.4) containing 4% paraformaldehyde (100-150ml) in case of light microscopy but 4% paraformaldehyde and 0.5% glutaraldehyde (100-150 ml) in case of immuno-electron microscopy. This experiment was reviewed by the committee of the Ethics on Animal Experiment in Yamaguchi University School of Medicine and carried out under the control of the guideline for Animal experiment in Yamaguchi University School of Medicine and The Law (No.105) and Notification (No.6) of the Government.

Immunohistochemistry for light microscopy

Olfactory bulbs were removed and immersed in the same fixative for 24 h. Then bulbs were soaked in cold 0.1 M phosphate buffer containing 30% sucrose (pH 7.4) until they sank. The bulbs were frozen in dry ice and frontally sectioned in a cryostat at a thickness of 30μm . Frozen sections were collected in ice-cold 0.02 M sodium phosphate buffered saline (PBS, pH 7.4) and rinsed twice with 0.02 M PBS (each time for 5 min). Sections were preincubated for 2h at 4℃ in PBS containing 20% normal goat serum (NGS), bleached for 1 h with 50% methanol containing 1.5% hydrogen peroxide and double -distilled water (DDW). After that, sections were washed three times (each time for 10 min) with 0.02 M PBS containing 0.3% Triton-X and 0.05% NGS. Then free floating sections were incubated for three days at 4℃ in Mab 213 diluted 1 : 1000 with 0.02 M PBS containing 0.05% NGS and 0.3% Triton-X. The monoclonal antibody designated as Mab 213 (clone 213-4.4) raised against TGFα, was purchased from Oncogene Science (Uniondale, NY). After three days, the primary antibody was washed out with 0.02 M PBS containing 0.05% NGS and 0.3% Triton -X. Then sections were incubated for 24 h at 4℃ in biotinylated anti-mouse goat IgG (Cappel) diluted 1 : 500 with 0.02 M PBS containing 0.05% NGS. After 24 h, sections were washed three times (each time for 10 min) with 0.02 M PBS containing 0.05% NGS followed by washing once with 0.02 M PBS (10 min). Sections were then incubated for 24 h at 4℃ in peroxidase-conjugated streptavidin (Vector) diluted 1 : 500 with 0.02 M PBS. Then, sections were washed three times (each time for 15 min) with 0.05 M Tris-HCl buffer (pH 7.6). After that, sections were reacted with 0.05 M Tris-HCl buffer containing 0.02% diaminobenzidine (DAB) and 0.6% nickel ammonium sulfate in presence of 0.006% hydrogen peroxide (nickel enhanced DAB reaction) for 10-15 min at room temperature. Then, the sections were washed once with 0.05M Tris-HCl buffer (15 min) followed by washing with 0.02 M PBS twice (each time for 15 min). Sections were then mounted onto glass slides in 1% gelatin/ saline, air dried, dehydrated with a graded series of ethanol, immersed in xylene and embedded in Entellan New. As a control, normal mouse serum was used instead of the

primary antibody. No glomerulus or receptor cell was immunostained in the control study.

AChE enzyme histochemistry for light microscopy

Adjacent sections were used for acetylcholinesterase (AChE) enzyme histochemistry. It was based on the method reported previously[6]. After washing with 0.1M maleate buffer (1.6 g/dl, pH 6.0) three times (each time for 10 min), sections were incubated for 1-2 h at 37℃ in a medium consisting of 36μM acetylthiocholine iodide (1.0 mg/dl), 5 μM $K_3Fe(CN)_6$ (0.16 mg/dl), 30μM $CuSO_4$ (0.47 mg/dl) and 50μ M sodium citrate $2H_2O$ (1.47 mg/dl) in 0.1 M maleate buffer (pH 6.0). They were then washed with 5 changes of 0.05M Tris-HCl (pH 7.6). Then sections were processed with 0.05 M Tris-HCl buffer (pH 7.6) containing 0.04% DAB, 0.3% nickel ammonium sulfate in presence of 0.003% hydrogen peroxide for 10-15 min at room temperature. After DAB reaction, sections were again washed three times with 0.05M Tris-HCl (pH 7.6), mounted onto glass slides in 1 % gelatin/saline, air dried, dehydrated with a graded ethanol series, cleared with xylene, and coverslipped with Entellan New.

Immunohistochemistry for electron microscopy

Serial sections of 0.5% glutaraldehyde fixed rat olfactory bulb were made at 30μm by vibratome (PL 1000). Then sections were collected in ice-cold 0.02 M sodium phosphate buffered saline (PBS, pH 7.4) and rinsed twice with 0.02 M PBS (each time for 5 min). Sections were preincubated for 2 h at 4℃ in 0.02 M PBS containing 20% normal goat serum (NGS). Then, sections were washed three times (each time for 10 min) with 0.02 M PBS containing 0.3% Triton-X and 0.05% NGS . After that, sections were rinsed once with 30% methanol in DDW for 5 min, bleached for 1 h with 50% methanol containing 1.5% hydrogen peroxide and DDW. Sections were then again rinsed once with 30% methanol in DDW for 5 min followed by washing once with 0.02 M PBS containing 0.3% Triton-X and 0.05% NGS for 5min. Sections were washed three times with 0.02 M PBS containing 0.05% NGS (each time for 10 min) and immersed in DDW containing 1.0% sodium borohydride for 1h. After washing three times with 0.02 M PBS containing 0.3% Triton-X and 0.05% NGS (each time for 15 min), the free floating sections were incubated for 3 days at 4℃ in Mab213 diluted 1 : 1000 with 0.02 M PBS containing 0.05% NGS and 0.3% Triton-X. After washing out of the primary antibody with 0.02M PBS containing 0.05% NGS and 0.3% Triton-X, sections were incubated for 24h at 4℃ in biotinylated anti-mouse goat IgG (Cappel) diluted 1 : 500 with 0.02 M PBS containing 0.05% NGS. Then sections were washed three times with 0.02 M PBS containing 0.05% NGS (each time for 10 min) followed by washing once with 0.02 M PBS (10 min). Sections were then incubated for 24h at 4℃ in peroxidase conjugated streptavidin (Vector) diluted 1 : 500 with 0.02 M PBS. Then, sections were washed three times (each time for 15 min) with 0.05 M Tris-HCl buffer (pH 7.6). After that, they were reacted with 0.05M Tris-HCl buffer containing 0.02% DAB and 0.6% nickel ammonium sulfate in presence of 0.006% hydrogen peroxide (nickel enhanced DAB reaction) for 10-15 min at room temperature. After the DAB reaction, sections were washed once with 0.05 M Tris-HCl buffer for 15 min followed by washing with 0.02 M PBS twice (each time for 15min). Then the immunostained sections were post-fixed for 1 h with 1% OsO_4 in 0.1 M phosphate buffer, dehydrated with a graded series of ethanol and infiltrated in propylene oxide. They were then put in a mixture of propylene oxide and Epok 812 (1 : 1) and flat-embedded on siliconized glass-slides in Epok 812. Sections were block-stained with 1% uranyl acetate for 1 h at a 70% alcohol dehydration state in order to enhance contrast for electron-microscopy. Serial ultrathin sections were made on an Reichert Jung ultramicrotome from trimmed areas of the embedded tissues which contained immunostained glomeruli and mounted onto copper grids. In order to obtain optimum relative contrast of the immunoreaction to the non-immunoreactive background, some ultrathin sections were observed under the electron-microscope (JEOL 200 CX) but others were observed after brief staining (for 90 sec) with 0.5% lead citrate to indicate

subcellular ultrastructure.

Terminology of rat nasal epithelium

Terminology of the rat nasal epithelium is based on the previously reported paper[1].

Results

The great majority of Mab 213-I olfactory receptor cells were distributed over dorsal portions of the nasal epithelium at the caudal end of nasal cavity (Fig. 1). Especially, such receptor cells were concentrated on the dorsal roof of the epithelium adjacent to the nasal septum and near the caudal end of the turbinate 1 (T1) (Fig. 2). Other scattered receptor cells were observed in the inferior part of the turbinate 6 (T6) and on the caudal walls of the cavities surrounded by turbinates 0 (T0) and 1 (T1), by the turbinates 2 (T2) and 3 (T3) and by turbinates 4 (T4) and 5 (T5). Some solitary Mab213-I receptor cells were also detected in the nasal septum. On the olfactory bulb, the Mab213-I glomeruli were found to be located along the posterior margin of main olfactory bulb with "a necklace pattern". One of the glomeruli was detected in the border of the main and accessory olfactory bulbs, belonging to "the modified glomerular complex" (MGC in Fig. 3). No immunoreactive glomerulus was observed at more rostral levels. Distribution of the Mab 213-I glomeruli was similar to that of the IAE patchy regions which consist of two distinct portions, the IAE-S and IAE-L portions. Immunostained glomeruli were found to correspond to the IAE-L portions which are adjacent to the IAE-S ones (Figs. 3, 4). In the toluidine-blue-stained sections, the Mab 213-I olfactory glomeruli were found to have dark nodules as well as other typical glomeruli do (Fig. 5). In immuno-electron microscopy, the immunoreactive glomeruli were found to have characteristic olfactory nerves containing "large dense-cored vesicles" of which sizes are 100-150 nm (Fig. 6B and 6C).

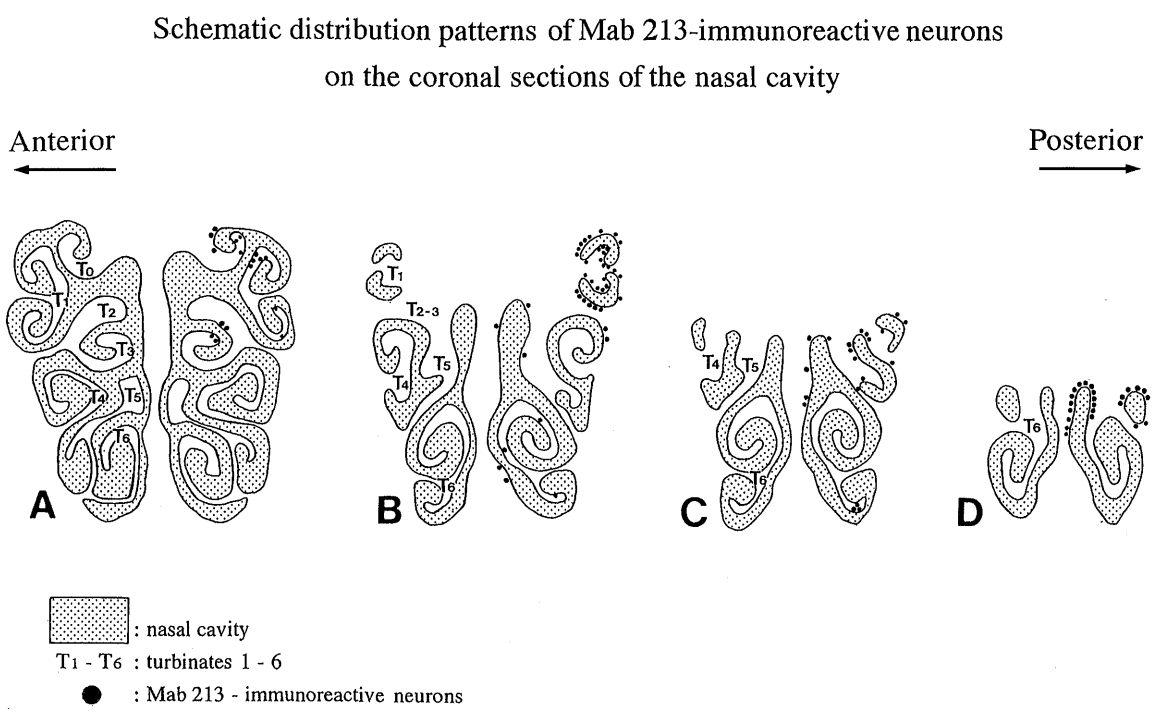

Fig 1. Schematic distribution pattern of the Mab 213-I neurons in the olfactory epithelium of normal adult rats. Four coronal sections (A-D) are arranged from anterior to posterior. The location of Mab 213-I neurons is indicated by black dots. Note that the great majority of neurons are concentrated in the epithelium on the dorsocaudal roof of cavity between the nasal septum and turbinate 6 (T_6) and near the caudal end of the turbinate 1 (T_1). T1-T6 : turbinates 1-6.

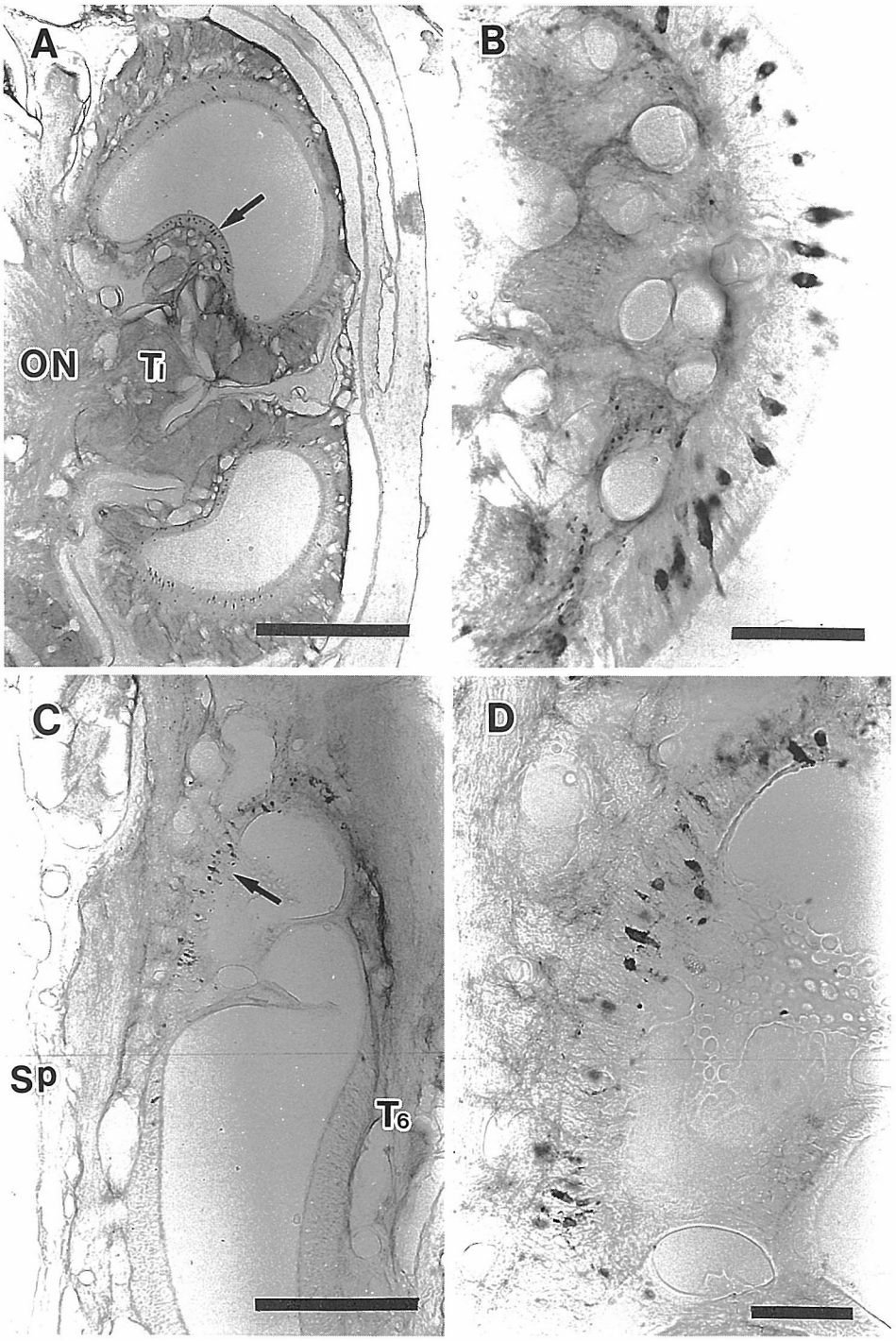

Fig 2. The Mab 213-I receptor neurons in the olfactory epithelium of a normal adult rat. **A**. Low magnification of photograph showing the Mab 213-I neurons near the caudal end of turbinate one (T_1). **B**. Higher magnification of the area indicated by an arrow in **A**. **C**. Low magnification of photograph showing the Mab 213-I receptor neurons present in the dorsocaudal roof of epithelium between the nasal septum and turbinate 6 (T_6). **D**. Higher magnification of the area indicated by an arrow in **C**.
Sp : nasal septum T1 : turbinate one T6 : turbinate 6 ON: olfactory nerve
Bars=500μm (A), 50μm (B, D), 250μm (C).

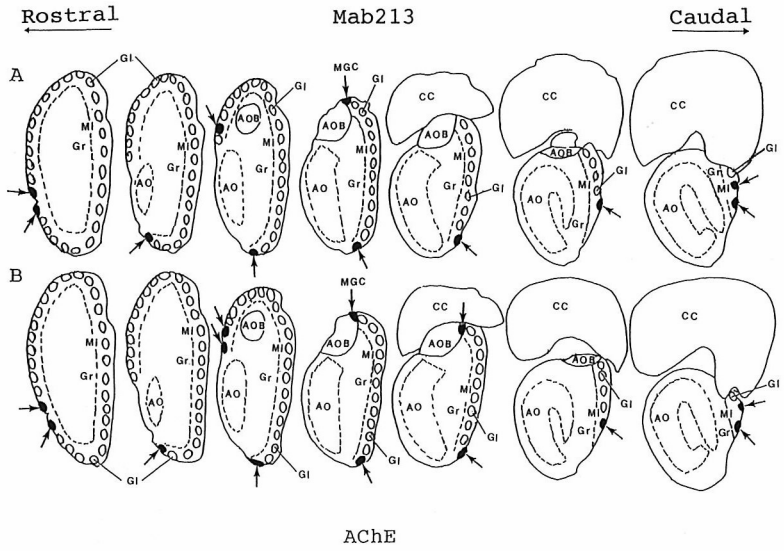

Fig 3. Schematic presentation showing the distribution of Mab 213-I (arrows in **A**) and acetylcholinesterase-reactive (arrows in **B**) glomeruli in the caudal levels of the olfactory bulb. Both groups are adjacent sections which are arranged from rostral to caudal. AO, anterior nuclear group ; AOB, accessory olfactory bulb; Gl, glomerular layer ; Mi, mitral cell layer ; CC, cerebral cortex ; MGC, modified glomerular complex.

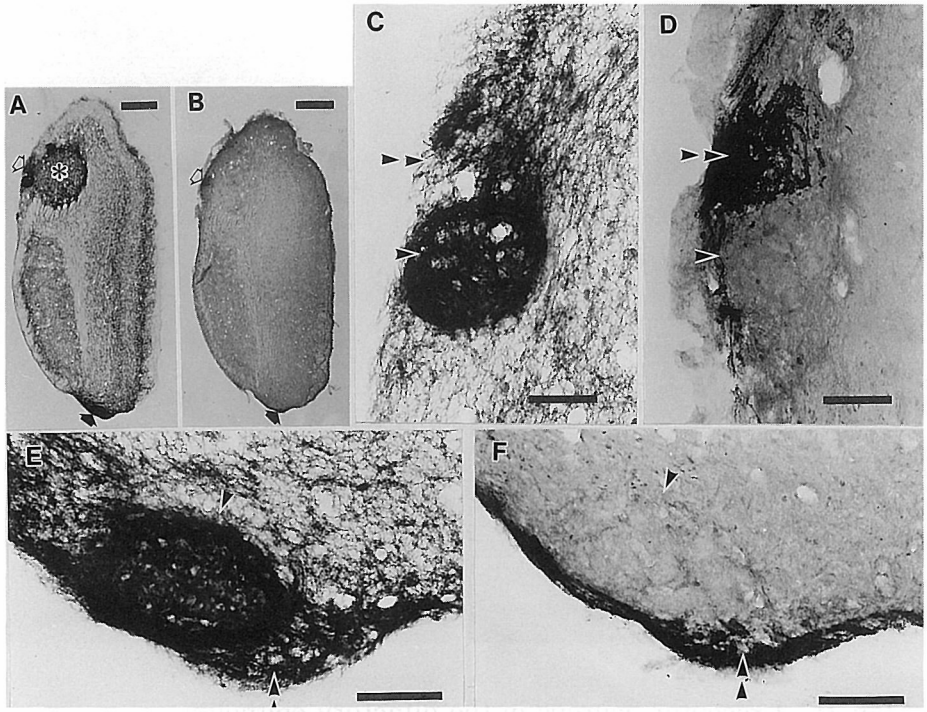

Fig 4. Light microscopic photographs showing relationship between the acetylcholinesterase-reactive (**A, C, E**) and Mab 213-I (**B, D, F**) glomeruli in adjacent sections. The regions indicated by clear arrows in **A** and **B** are enlarged in **C** and **D**, respectively, while the ones indicated by solid arrows in **A** and **B** are enlarged in **E** and **F**, respectively. An asterisk indicates acetylcholinesterase-stained accessory olfactory bulb. Note that strongly reactive IAE portion (IAE-S : arrow heads in **C** and **E**) corresponds to the immunonegative glomeruli (arrow heads in **D** and **F**) while the less reactive IAE portion (IAE-L : double arrow heads in **C** and **E**) corresponds to the Mab 213-I glomerulus (double arrow heads in **D** and **F**).
Bars=500μm (A, B), 200μm (C, D), 100μm (E, F).

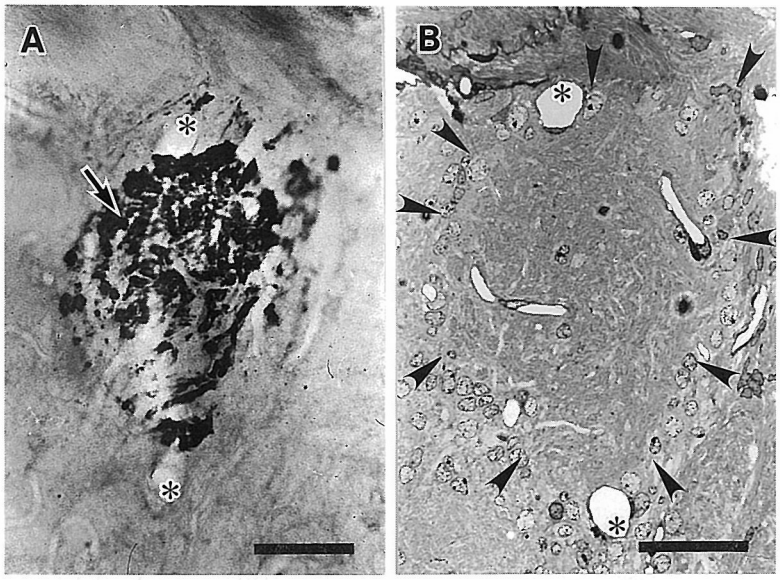

Fig 5. A. The Epok-embedded Mab 213-I glomerulus (arrow) in the modified glomerular complex. B. The same semithin section (1μm) stained by toluidine blue. Asterisks indicate the blood vessels as landmarks. The glomerulus corresponding to the Mab 213-I one shows a prominent dark nodule in the toluidine-blue-stained section (surrounded by arrow heads in B).
Bars=50μm (A, B).

Axon terminals of such olfactory nerves were sometimes observed to make synaptic contacts with some dendrites (Fig. 6C). Although the immunoreaction appeared to be diffusely present not only in the axonal cytoplasm but also on the large dense-cored vesicles (Fig. 6B and 6C), it was difficult to determine the exact localization of immunoreaction in the present "pre-embedding" method for immuno-electron microscopy.

Discussion

The present study has clearly provided data for structural features of a subset of olfactory neurons and glomeruli immunostained with Mab 213. In the nasal cavity, the great majority of Mab 213-I olfactory neurons were concentrated in the dorsocaudal end of the olfactory epithelium, especially on the dorsal roof adjacent to the nasal septum and near the caudal end of the turbinate 1 (T1). The Mab 213-I olfactory glomeruli were found to form a necklace pattern surrounding the caudal end of the olfactory bulb and correspond to the IAE-L which are adjacent to the IAE-S. The most striking finding in the present study was that the Mab 213-I glomeruli are characterized by the presence of dark nodules and olfactory nerves rich in large dense-cored vesicles.

The monoclonal antibody designated as Mab 213 was raised against TGFα, which is an acid- and heat-stable 50 amino acid protein of 5500 MW originally found in rodents and humans and secreted by a variety of transformed cells and tumors, embryonic cells, and some normal adult cells [7]. It has been reported that, as a result of testing five different anti-TGFα antibodies purchased or gifted from other companies (three from Research & Diagnostic Antibodies, Santa Cruz Biotechnology and Research Diagnostics) or laboratories (two from Dr. L. Gentry), none of these can label any olfactory neurons or glomeruli[5]. Furthermore, Mab 213 does not label this neuronal subset in fresh-frozen sections of the olfactory epithelium or bulb[5]. Hence it is unlikely that the neuronal antigen that is recognized by Mab 213 is TGFα but, rather, some other structurally related epitope that is generated de novo as a consequence of fixation with cross-linking agents[5]. Therefore, the present discussion will mainly focus on morphological aspects of the stained structures.

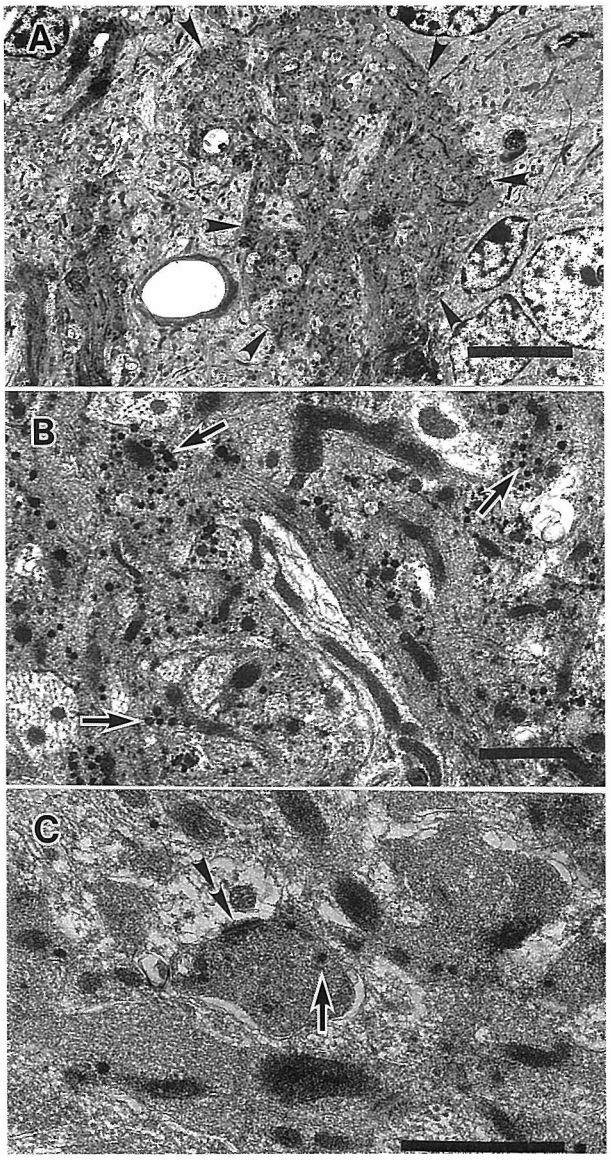

Fig 6. Electron micrographs of the Mab 213-I glomerulus demonstrated in Fig.5. **A**. Lower magnification of the immunoreactive glomerulus (surrounded by arrow heads). **B**. A plenty of large dense-cored vesicles (arrows) which are characteristics of the olfactory nerves in the Mab 213-I glomerulus. **C**. An axon terminal containing large dense-cored vesicles (arrow) which makes a synaptic contact (double arrow heads) to some dendritic profile.
Bars=5 μm (A), 1 μm (B, C).

The present results concerning the distribution of Mab 213-I olfactory receptor cells almost coincide with the previous data of Ring et al[5]. Both confirm the topographic organization between the dorsocaudal end of the nasal epithelium and the Mab 213-I necklace glomeruli which are located at the caudal end of the bulb. Interestingly, presence of similar topographic organization has already been shown in the hPAX-P2 immunoreactive primary olfactory system[1].

The IAE region had sometimes been confused with the "atypical glomerulus" which was incorrectly described to have large dense-cored vesicles and no dark nodule[8,9]. As to the confusion, Shinoda et al[2]. reported that the IAE region is heterogeneous and composed of, at least, three distinct glomeruli (or glomerulus-like parts): the IAE-S, IAE-L/hPAX-P2, and IAE-L/non-hPAX-P2 glomeruli. The hPAX-P2 immunoreactive glomeruli were clarified to contain dark nodules in light microscopy and large dense-cored vesicles in electron-microscopy[3]. Recently the IAE-S glomeruli were demonstrated to be lack of dark nodules which are prominent bulks of the primary olfactory nerves and to have no large dense-cored vesicles[10]. Therefore, original description of the two characteristics of "atypical glomerulus" by Zheng et al[8,9]. is thought to reflect separate features in two different portions of the IAE region ; a lack of dark nodules belongs to characteristics of the IAE-S, while the presence of large dense-cored vesicles to those of the IAE-L/hPAX-P2. Since the dark nodules are regarded to be essential for typical olfactory glomeruli, it is appropriate to use the term "atypical glomerulus" as the IAE-S. The present results clearly showed that the Mab 213-I glomeruli are adjacent to the IAE-S and have dark nodules and nerve terminals containing large dense-cored vesicles, strongly suggesting that they are identical with the IAE-L/hPAX-P2 glomeruli, namely the Shinoda's "necklace glomeruli". This speculation is also supported by the present evidence regarding the distribution of Mab 213-I olfactory receptor cells in the nasal epithelium which resembles that of the hPAX-P2 immunoreactive receptor cells ; both are concentrated in the dorsocaudal wall of the nasal cavity. The definitive conclusion, however, requires the double-immunostaining using Mab 213 and anti-hPAX-P2

antibodies.

It has recently been reported that the Mab 213-I glomeruli are strongly labeled with antibody against synaptophysin and neural cell adhesion molecule (NCAM), but only weakly stained with the antibody to the olfactory marker protein (OMP) [5] which has been regarded as essential to the primary olfactory system[11]. Ring et al[5]. have described that there are another two distinct necklace-typed glomeruli in the caudal free margin of the olfactory bulb. One type is labeled with a monoclonal antibody 2C6 (not with Mab 213), being reported to be only weakly stained with anti-OMP antibody too, while the other type is not labeled with Mab 213 or 2C6 antibody, being reported to be devoid of OMP and very weakly stained with NCAM[5]. Necklace-typed glomeruli have also been pointed out to be very unique in its second messenger system. Most of the primary olfactory system has been known to utilize the cAMP as a second messenger[12], whereas the "necklace olfactory system" has been shown to contain the PDE2, suggesting that it is regulated by cGMP signal transduction pathway instead of the cAMP pathway [4]. In the previous study, another necklace -typed glomeruli which belong to the IAE-L but non-hPAX-P2 immunoreactive glomeruli were also reported to be present near the IAE -L/hPAX-P2 glomeruli[2]. The detailed analysis, however, has yet to be performed.

It is of importance that one of the necklace glomeruli which were stained with Mab 213 or anti-hPAX-P2 or -PDE2 antibody, has been detected in the modified glomerular complex which was previously reported to be involved in rat suckling behavior[1,13,14]. The suckling behavior, however, cannot be deleted by a local lesion of the modified glomerular complex in the rat[15] or rabbit[16]. It therefore seems likely that the modified glomerular complex is not a special site relevant to the suckling behavior, but just one member of the Mab 213-I or Shinoda's "necklace glomeruli" which are, as a whole, probably associated with some pheromone-like olfactory information inducing the rat-pap suckling behavior [1,2,4,5]. Other kinds of necklace glomeruli which have recently been reported in the caudal free margin of the bulb[4,5] might also be minimum functional units which process distinct olfactory information from other unique primary olfactory systems.

Acknowledgments

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References

1) Shinoda, K., Shiotani, Y., and Osawa, Y.: "Necklace olfactory glomeruli" form unique components of the rat primary olfactory system. *J. Comp. Neurol.*, **284** : 362-373, 1989.

2) Shinoda, K., Ohtsuki, T., Nagano, M., and Okumura, T.: A possible functional necklace formed by placental antigen X-P2-immunoreactive and intensely acetylcholinesterase-reactive (PAX/IAE) glomerular complexes in the rat olfactory bulb. *Brain Res.*, **618** : 160-166, 1993.

3) Ohtsuki, T., Shinoda, K., Mori, S., and Shiotani, Y. : Ultrastructure and postnatal development of "necklace olfactory glomeruli"of the rat. : *Acta Anat. Nipponica (Abstr.)*, **66** : 287, 1991.

4) Juilfs, D. M., Fulle, H. J., Zhao, A. Z., Houslay, M. D., Garbers, D. L., and Beavo, J. A. : A subset of olfactory neurons that selectively express cGMP -stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. : *Proc. Natl. Acad. Sci. USA.*, **94** : 3388-3395, 1997.

5) Ring, G., Mezza, R. C., and Schwob, J. E. : Immunohistochemical identification of discrete subsets of rat olfactory neurons and the glomeruli that they innervate. *J. Comp. Neurol.*, **388** : 415-434, 1997.

6) Tago, H., Kimura, H., and Maeda, T. : Visualization of detailed acetylcholinesterase fiber and neuron staining in rat brain by a sensitive histochemical procedure. *J. Histochem. Cytochem.*, **34** :

1431-1438, 1986.
7) Deryck, R. : Transforming growth factor -α : structure and biological activities. *J. Cell Biochem.*, **32** : 203-204, 1986.
8) Zheng, L. M., Ravel, N., and Jourdan, F. : Topography of centrifugal acetylcholinesterase-positive fibers in the olfactory bulb of the rat: Evidence for original projections in atypical glomeruli. *Neuroscience*, **23** : 1083-1093, 1987.
9) Zheng, L. M., and Jourdan, F. : Atypical olfactory glomeruli contain original olfactory axon terminals : An ultrastructural horseradish peroxidase study in the rat. *Neuroscience*, **26** : 367-378, 1988.
10) Okumura, T., Shiotani, Y., Nagano, M., Sasaki, H., and Shinoda, K. : Analysis of intensely acetylcholinesterase-reactive glomerulai-like regions of the rat olfactory bulb. *Acta· Anat. Nipponica (Abstr.)*, **69** : 529, 1994.
11) Monti-Graziadei, G. A., Margolis, F. L., Harding, J. W. and Graziadei, P. P. C. : Immunocytochemistry of the olfactory marker protein. *J. Histochem. Cytochem.*, **25** : 1311-1316, 1977.
12) Brunet, L. J., Gold, G. H., and Ngai, J. : General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron*, **17** : 681-693, 1996.
13) Greer, C.A., Stewart, W.B., Kauer, J. S., and Shepherd, G.M. : Topographical and laminar localization of 2-deoxyglucose uptake in rat olfactory bulb induced by electrical stimulation of olfactory nerves. *Brain Res.*, **217** : 279-293, 1981.
14) Teicher, M. H., Stewart, J. S., Kauer, J. S., and Shepherd, G. M. : Suckling phenomenon stimulation of a modified glomerular region in the developing rat olfactory bulb revealed by the 2-deoxyglucose method. *Brain Res.*, **194** : 530-535, 1980.
15) Risser, J. M., and Slotnick, B. M. : Suckling behavior in rat pups with lesions which destroy the modified glomerular complex. *Brain Res. Bull.*, 19 : 275-281, 1987.
16) Hudson, R., and Distel, H. : Regional autonomy in the peripheral processing of odor signals in newborn rabbits. *Brain Res.*, 421 : 85-94, 1987.