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ODORANT RECEPTOR PROTEINS IN THE MOUSE MAIN OLFACTORY EPITHELIUM AND OLFACTORY BULB

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Abstract—In the mouse, odorant receptor proteins (ORs) are G-protein-coupled receptors expressed in mature olfactory sensory neurons (OSNs) of the main olfactory epithelium (MOE). ORs mediate odorant reception at the level of the OSN cilia. Most of the ~1100 OR genes in the mouse genome are expressed, at the RNA level, in mature OSNs. The literature on antibodies against ORs is limited, and most reports are with antibodies that are not commercially available. Here we have screened 40 commercial antibodies against human and mouse ORs by immunofluorescence staining of coronal cryosections of the MOE of 21-day-old C57BL/6J mice. Various methods of antigen retrieval were tested. Of the 19 antibodies raised against human ORs, three yielded a consistent immunoreactive signal in the mouse MOE; of these three, two appeared to cross react against one or more, unknown, mouse ORs. Of the 21 antibodies raised against mouse ORs, six yielded a consistent immunoreactive signal in the mouse MOE; of these six, two also stained specific glomeruli in the olfactory bulb. Antibody specificity could be validated with gene-targeted mouse strains in the case of three ORs. The number of OSNs immunoreactive for the MOR28/Olfr1507 antibody is greater in C57BL/6J than in 129S6/SvEvTac wild-type mice. Taken together, our results are encouraging: 20–30% of these commercially available antibodies are informative in immunohistochemical analyses of the mouse MOE. The commercial availability of these antibodies should facilitate the study of OR proteins in the MOE and the olfactory bulb, and the replicability of results in the literature.

Key words: olfactory receptor, olfactory sensory neuron, glomerulus, antibody, immunofluorescence, antigen retrieval.

INTRODUCTION

In mammals such as mouse and rat, odorants are detected in the main olfactory epithelium (MOE) by olfactory sensory neurons (OSNs) via odorant receptors (ORs), which are G-protein-coupled seven-transmembrane proteins (Buck and Axel, 1991). In the mouse, there are 1099 OR genes with an intact open reading frame, of which 1087 are expressed at the RNA level in OSNs (Saraiva et al., 2015).

Due to the difficulty in raising antibodies against G-protein-coupled receptors, there have been relatively few reports about OR proteins in the literature. Initial studies with antibodies against rat ORs suggested that the antigen is localized to the cilia of OSNs in the rat MOE (Koshimoto et al., 1992; Krieger et al., 1994). *In situ* hybridization against OR RNAs in mouse revealed that OR gene expression is restricted to OSNs within discrete regions of the MOE called zones (Ressler et al., 1993; Miyamichi et al., 2005). Gene-targeted mice enabled the visualization of entire OSNs expressing a given OR by targeted integration of a cassette that affords cotranslation of the OR with a marker such as taulacZ via an internal ribosome entry site (IRES) (Mombaerts et al., 1996). Axons of OSNs expressing the same OR coalesce into two or a few glomeruli per mouse olfactory bulb, which reside in highly restricted regions (Ressler et al., 1994; Mombaerts et al., 1996; Zapiec and Mombaerts, 2015). Replacement of the OR coding region showed that the OR does not only mediate odorant detection (Bozza et al., 2002), but is also a determinant of where in the olfactory bulb the axons coalesce and form glomeruli (Mombaerts et al., 1996; Feinstein et al., 2004; Feinstein and Mombaerts, 2004).

The widespread success of the gene-targeted approach of the OR-IRES-marker design may have overshadowed or diminished efforts to raise and characterize OR antibodies. But these gene-targeted mutations are designed to visualize the OSNs (including their cilia, dendrite, cell body, axon, and axon terminals) that express a given OR, and do not enable the visualization of the subcellular localization of the OR protein within the OSNs. An attempt at creating an OR:GFP C-terminal fusion protein by gene targeting resulted, for reasons that remain unclear, in altered positions of the corresponding glomeruli in the olfactory bulb (Feinstein et al., 2004).

Antibodies against mouse ORs revealed that OR proteins are present not only in OSN cilia, dendrite, and cell body but also within the axon and axon terminals

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Abbreviations: aa, amino acid; AR, antigen retrieval; EDTA, ethylenediaminetetraacetic acid; GFP, green fluorescent protein; IRES, internal ribosome entry site; MOE, main olfactory epithelium; OMP, olfactory marker protein; OR, odorant receptor; OSN, olfactory sensory neuron; PBS, phosphate-buffered saline; PFA, paraformaldehyde; SDS, sodium dodecyl sulfate.

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(Barnea et al., 2004; Strotmann et al., 2004), thus supporting the notion that the OR protein is involved in axonal wiring of OSNs.

A quarter of a century after the discovery of mammalian OR genes (Buck and Axel, 1991), there are still few papers on immunofluorescence staining of the mouse MOE and olfactory bulb with OR antibodies that are commercially available. There are numerous antibodies against ORs on the market, but they remain largely uncharacterized. The signal yielded by such antibodies can be affected by the high sequence homology between ORs, by post-translational modifications, by protein folding, by protein–protein interactions, and by fixation crosslinking. To increase the accessibility of the antigen to the antibody, antigen retrieval can be used to break some of the crosslinks formed during fixation and to alter the protein re-folding following heat denaturation (Shi et al., 2001; Emoto, 2005). Epitope unmasking through antigen retrieval can allow for a more accurate detection, and therefore representation, of the antigen.

Here, we describe our screening of 40 commercially available antibodies against human and mouse ORs in the mouse MOE using various antigen retrieval techniques, in coronal 12-μm sections of the MOE of 21-day-old C57BL/6J mice. We obtained reliable immunoreactive signals with two antibodies against human ORs (due to presumed crossreactivity with mouse ORs) and six antibodies against mouse ORs in the mouse MOE. Of these six, two antibodies also stained specific glomeruli in the olfactory bulb.

EXPERIMENTAL PROCEDURES

Mice

Mice were 21 days old. Wild-type mice were C57BL/6J or 129S6/SvEvTac. Our gene-targeted strains (Feinstein et al., 2004) are publicly available from The Jackson Laboratory (Bar Harbor, ME, USA): mI7-IRES-tauGFP (https://www.jax.org/strain/006664), and M50-IRES-GFP-IRES-taulacZ (https://www.jax.org/strain/006686). The gene-targeted strain MOR28-IRES-gap-GFP (Serizawa et al., 2000) is publicly available from the RIKEN BioResource Center (Tsukuba, Japan) (https://www2.brc.riken.jp/lab/animal/detail.php?brc_no=RBRC02928). Mice were maintained in specified pathogen-free conditions in individually ventilated cages of the Tecniplast green line. Mice received *ad libitum* gamma-irradiated ssniff V1124-727 (ssniff, Soest, Germany). Nesting, bedding, and enrichment were provided as nestpak, Datesand Grade 6 (Datesand, Manchester, United Kingdom). Mouse experiments were performed in accordance with the German Animal Welfare Act, European Communities Council Directive 2010/63/EU, and institutional ethical and animal welfare guidelines of the Max Planck Research Unit for Neurogenetics. All efforts were made to minimize the number of animals used and their suffering.

Immunohistochemical staining

Tissue preparation. Mice were anesthetized by injection of ketamine HCl and xylazine (120 mg/kg and 5 mg/kg body weight, respectively) and perfused with 5 ml ice-cold phosphate-buffered saline (PBS), followed by 15 ml 2% paraformaldehyde in PBS (PFA). The mouse heads were dissected, postfixed in 2% PFA for 2 h at 4 °C, and decalcified in 0.45 M EDTA in PBS overnight at 4 °C. Samples were cryoprotected in, successively, 15% and 30% sucrose in PBS at 4 °C, frozen in O.C.T. Compound (Tissue-Tek), sectioned at 12 μm with a Leica CM3500 cryostat, and collected onto glass slides. Alternative fixation methods were also tested; perfusion with 4% PFA resulted in poorer antigenicity, and immersion fixation in 2% PFA for 2 h without perfusion did not yield improved antigenicity.

Antigen retrieval. Various antigen retrieval steps were tested to optimize visualization of the target protein. These included a 10-min incubation in 2 M HCl at room temperature, or 10 min in ice-cold 100% methanol followed by 0.5% sodium dodecyl sulfate (SDS) in PBS at room temperature. Antigen retrieval with a pressure cooker (2100 Retriever; BioVendor) was carried out with 10 mM citric acid pH 6.0 and 10 mM Tris pH 9.0 with or without 0.05% Tween 20 and 2 mM EDTA. After heating in a pressure cooker to 121 °C, slides were left to cool for 2 h in the retrieval buffer. Additionally, PBS washes were tested with 0.05% Tween 20 or 0.2% Triton X-100, and incubation with antibody was tested for various times during a period of 1–4 days, with and without 0.1% Triton X-100. Best results were obtained with PBS washes and incubation with antibody diluted with 0.1% Triton X-100. There was no obvious difference in antigen detection with different incubation times.

Immunohistochemistry. Slides were washed 3 × 10 min with PBS, and, if necessary, antigen retrieval was carried out and the slides were again washed. Slides were then blocked with 10% normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) or normal donkey serum (Millipore, Darmstadt, Germany) in PBS for 1 h at room temperature. After blocking, slides were incubated with the primary antibodies in 1% normal goat serum or normal donkey serum with 0.1% Triton X-100 in PBS overnight at 4 °C. Commercial rabbit antibodies against ORs were tested at dilutions of 1:200, 1:500, and 1:2000. The following antibodies against human ORs were obtained from Osenses (Keswick, SA, Australia): OR11H4, OR1B8, OR1D4, OR1E1, OR1L8, OR1M1, OR1N2, OR2A4, OR2D3, OR2F1, OR2H1, OR2K2, OR2T1, OR2V1, OR2W1, OR4C11, OR51E1, OR8A1. The following antibodies against mouse ORs were obtained from Osenses: Olfr2, Olfr6 (x2; different peptide sequences), Olfr15, Olfr16, Olfr24, Olfr56, Olfr151, Olfr156, Olfr362, Olfr412, Olfr521, Olfr545, Olfr552, Olfr554, Olfr685, Olfr749, Olfr831, Olfr1507. We also tested sheep OR51E1 and chicken Olfr73 antibodies (Osenses, Keswick, SA, Australia), and rabbit Olfr1303 antibody (Life Technologies, Waltham, MA, USA). Mature OSNs were visualized with goat OMP antibodies (olfactory marker protein; 1:1000; Wako Chemicals, Neuss, Germany), and green fluorescent