A novel role for scavenger receptor B1 as a contributor to the capture of specific volatile odorants in the nasal cavity

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ABSTRACT

Class B scavenger receptors, scavenger receptor B1 (SR-B1) and cluster of differentiation 36 (CD36), are broadly expressed cell-surface proteins and are believed to serve as multifaceted players in lipid and lipoprotein metabolism in mammals. Because of its ability to recognise distinct odour-active volatile compounds and its presence in murine olfactory epithelium, CD36 has recently emerged as a participant in the detection of odorants within the nasal cavity. However, there have been no attempts to assess whether SR-B1 has such a role. In this study, we performed a cell-free in-vitro assay utilising a peptide mimic of the receptor, and demonstrated that SR-B1 could recognise aliphatic aldehydes (e.g., tetradecanal), a distinct class of volatile odorants, as potential ligands. By reverse transcription-polymerase chain reaction and western immunoblot analyses, we detected the expression of SR-B1 mRNA and protein, respectively, in mouse olfactory tissue. Finally, we immunohistochemically mapped the distribution of SR-B1 in the surface layer of olfactory epithelium in vivo, which is the first line of odorant detection. These findings uncover a novel role for SR-B1 as a contributor to the capture of specific odorants in the nasal cavity of mammals.

Class B scavenger receptors are cell-surface proteins, characterised by two predicted transmembrane spans, broad expression patterns and the ability to recognise diverse ligands (13). Of these, scavenger receptor B1 (SR-B1) was initially identified as comprising 509 amino-acid residues (5). An earlier study has showed that SR-B1 is expressed in several tissues including the liver and adipose tissues, and can serve as a receptor for low-density lipoprotein (LDL), acetylated LDL, oxidised LDL (oxLDL) and maleylated bovine serum albumin (BSA) (2). Later, SR-B1 was found to recognise several other substances such as high-density lipoprotein (HDL) (1) and anionic phospholipids (15). These findings suggested that SR-B1 primarily participates in lipid and lipoprotein metabolism in internal organs (36). As studied extensively, it functions as a physiologically relevant HDL receptor to mediate the selective delivery of HDL-cholesterol (i.e., HDL-cholesteryl ester) to the liver and steroidogenic tissues (1, 13, 20).

Another member of class B scavenger receptors, cluster of differentiation 36 (CD36), which comprises 472 amino-acid residues, is also a multifaceted and multifunctional player in lipid and lipoprotein metabolism (13, 27, 28). CD36 is known to share with SR-B1 the ability to recognise distinct ligands,

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including oxLDL (13). Intriguingly, Podrez and co-workers showed that: (i) certain forms of oxidised glycerophosphocholine (oxGPC) (i.e., Those having a terminal γ-hydroxy(or oxo)-α,β-unsaturated carbonyl on the sn-2 acyl group, hereafter referred as oxGPC$_{SRB}$), which can occur on the surface of LDL particles in an oxidative environment, bind with high affinity to full-length CD36 (24), (ii) the site for oxGPC$_{SRB}$ binding lies within the extracellular loop region of the receptor (amino acid residues 118–182) (9, 14), and (iii) a synthetic peptide comprising residues 154–168 effectively inhibits the binding of fl-oxLDL to the receptor (14). They also found that (i) oxGPC$_{SRB}$ species inhibit oxLDL binding to full-length SR-B1_F (3) and (ii) smaller unilamellar vesicles containing oxGPC$_{SRB}$ bind directly to the amino acid region 183–205 located in the extracellular loop of the receptor (9). These findings suggest that oxGPC$_{SRB}$ species occurring on the oxLDL surface serve as structural moieties essential for the particle binding to CD36 and SR-B1 and that only a short amino acid region is sufficient for oxLDL recognition in either of the receptors.

With regard to CD36, we have shown that fluorescently labelled oxLDL (fl-oxLDL) binds specifically and saturably to synthetic peptides of about 20 amino acids containing residues 154–168 of this receptor (16, 29–31), supporting the idea that only a short segment of this receptor is responsible for the binding of such large particles. It was also found that oxGPC$_{SRB}$ species, like 1-(palmitoyl)-2-(5-keto-6-octenediyl)phosphatidylcholine (KodiA-PC, one of the most potent lipid ligands of CD36), inhibited the binding of fl-oxLDL to the CD36 peptides (16, 30, 31), confirming that the peptides bind oxLDL particles by recognising their oxGPC$_{SRB}$ moiety. A novel finding of our studies using the CD36 peptides was that aliphatic aldehydes such as tetradecanal, a distinct class of volatile odors, were capable of inhibiting fl-oxLDL binding to the mimics (i.e., were identified as potential CD36 ligands) (32, 33, 35). Furthermore, others and we have discovered that CD36 is expressed by a population of olfactory sensory neurons and is abundantly present in the olfactory ciliary layer in mice (17, 22, 38). These recent findings led us to propose that CD36 participates in the capture and detection of specific odors in the nasal cavity of mammals. Given its ability to recognise oxLDL/oxGPC$_{SRB}$ and its broad expression pattern (13, 20), it is possible that SR-B1 exists and plays a role similar to CD36 in the sensory organ. However, there have been no attempts to address these issues till date.

The objective of this study was to assess whether SR-B1 participates in the recognition and capture of specific odorants in the nasal cavity of mammals. Our first aim was to obtain in vitro evidence for the ability of SR-B1 to recognise odour-active volatile compounds. For this, we designed and developed a short SR-B1 peptide that could specifically bind fl-oxLDL and found that certain aliphatic aldehydes (e.g., tetradecanal) inhibited binding of the labelled lipoprotein to the peptide, suggesting that SR-B1 can recognise certain volatile odorants as potential ligands. Our second aim was to determine whether SR-B1 exists and functions in the mammalian olfactory system. To this end, we performed reverse transcription-polymerase chain reaction (RT-PCR) and electrophoresis/western immunoblot analyses for samples obtained from the nasal tissue of mice and showed SR-B1 expression in the tissue. Furthermore, we immunohistochemically demonstrated the presence of SR-B1 in the surface layer of olfactory epithelium. Our present findings suggest that SR-B1 contributes to the capture of volatile odorants in the nasal cavity of mammals.

MATERIALS AND METHODS

Materials. Human oxLDL with a thiobarbituric acid-reactive substance value of ~30 nmole of malondialdehyde/mg of protein was obtained from Biomedical Technologies (Stoughton, MA, USA). Alexa Fluor® 488 reactive dye was obtained from Thermo Fisher Scientific Japan (Tokyo). Bovine serum albumin (BSA, fatty acid free), Z-11-hexadecenal, E-2-tridecenal, hexanal and benzaldehyde were obtained from Sigma-Aldrich Japan (Tokyo). Dipalmitoylphosphatidylcholine (DPPC) and KODI-A-PC were purchased from Cayman Chemical (Ann Arbor, MI, USA). n-Octanal (hereafter referred to as octanal), decanal, dodecanal, tetradecanal, hexadecanal, octadecanal and citral (a mixture of cis- and trans- isomers) were from Tokyo Chemical Industry (Tokyo).

Preparation of mimic peptides for class B scavenger receptors. An oligopeptide containing amino acid residues 149–168 of human CD36 with biotin at the N-termi

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Preparation of fl-oxLDL. The procedure for labelling of human oxLDL with the fluorescent dye AlexFluor® 488 was the same as described previously (32, 33, 35). The fl-oxLDL particles were stored in a light shielding vial at 4°C until use.

Pre-treatment of streptavidin-coated plates with synthetic peptides and evaluation of fl-oxLDL binding to the peptides. The wells of a 96-well plate pre-coated with streptavidin (Thermo Fisher Scientific Japan) were pre-washed thrice with phosphate buffered saline (PBS; 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 136 mM NaCl and 2.7 mM KCl; pH 7.4) containing 0.4% w/v bovine serum albumin (hereafter referred to as PBS-BSA). A 0.05-mL aliquot of PBS-BSA alone or PBS-BSA containing synthetic peptides at a concentration of 5 μM were added to each well of the plate. Subsequently, the plate was incubated at an ambient temperature for 1 h with gentle shaking. After washing the well-plate thrice with PBS containing 0.05% w/v BSA, 0.05 mL of PBS-BSA containing fl-oxLDL at a concentration of 10 μg protein/mL in the presence or absence of non-labelled oxLDL (0.48 mg/mL) was added to each well. Next, the plate was incubated in the dark at an ambient temperature for 1 h with gentle shaking. After incubation, the wells were initially washed thrice with 0.2 mL of PBS, followed by addition of 0.1 mL PBS. The fluorescence in each well was measured using a plate reader (PowerScan; Dainippon Sumitomo Pharma, Osaka, Japan) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm.

Determination of dissociation constant ($K_d$) and receptor density ($B_{max}$). Wells pre-treated with 5 μM of synthetic peptides were exposed to 0.05 mL of PBS-BSA containing various concentrations of fl-oxLDL, and the assay plate was incubated as described above. After harvesting the assay mixtures, the fluorescence in each well was measured as described above. The fluorescence in each well was obtained by subtracting the mean background of the wells incubated with PBS-BSA alone (PBS-BSA without fl-oxLDL). Specific binding of fl-oxLDL was determined by subtracting the background binding to peptide-untreated control wells at each concentration of fl-oxLDL. $K_d$ and $B_{max}$ were determined by fitting the progress curve to the theoretical curve obtained using Eq. 1 as follows, using a commercially available software (Prism® version 7.0d; GraphPad, San Diego, CA, USA):

$$B = B_{max} \frac{[L]}{(K_d + [L])}$$

(eq. 1)

In the above equation, $B$ is the amount of fl-oxLDL bound to the immobilised SR-B1 mimic in a well, and $[L]$ is the concentration of fl-oxLDL (23). The amount of fl-oxLDL bound to the peptide was assessed by comparing with the fluorescence of wells that received 0.1 mL of PBS containing fl-oxLDL particles at known concentrations.

$fl$-oxLDL binding/inhibition assays. Working solutions of chemicals were prepared as follows: solutions of the test lipids dissolved in ethanol were diluted with ethanol. A 5-μL aliquot of the diluted lipids was placed in a 1.5-mL microcentrifuge tube, and 95 μL of a concentrated solution of PBS (1.05 × concentrate) containing 0.42% (w/v) BSA, 42 μM of butylated hydroxytoluene, 210 μM diethylenetriamine penta-acetic acid, and 10.5 μg protein/mL of fl-oxLDL was added, and used in the fl-oxLDL-binding/inhibition assay.

Untreated wells and wells pre-treated with 5 μM of synthetic peptides received 0.05 mL of working solutions. The plates were then incubated for 1 h at an ambient temperature. After the incubation, the fluorescence of each well was measured as described above. The fluorescence values for wells pre-treated with peptides were obtained by subtracting the mean fluorescence value of peptide-untreated controls. Prism® software was used to determine the concentration required for 50% inhibition of binding (IC₅₀).

Animals. Adult C57/BL6 mice (Shimizu Laboratory Supplies, Kyoto, Japan) were utilised in this study. The usage of mice in this study was approved by the Kyoto University Animal Experimentation Committee, and the experiments were in accordance with the Kyoto University Guidelines for the Ethical Treatment of Laboratory Animals.

RT-PCR analysis. Total RNA was extracted from the nasal mucosa and liver of mice using the RNaseasy Mini Kit (QIAGEN K. K.-Japan, Tokyo). RT-PCR was performed as described previously (25). Primer sets (synthesised by Life Technologies Japan) were used as follows: 5’-TCAAGCAGCAGTGCTCA-3’ and 5’-GAGGATTCCGGTGTATGAA-3’ (for SR-B1 mRNA), 5’-CCCTGTGCTGCTCACC-3’ and 5’-GCACGATTTCCCTCTCAG-3’ (for β-actin mRNA). PCR products were separated on 2% agarose gel, and visualised using SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific Japan).

Western immunoblot analysis. Tissue pieces from
the nasal mucosa and liver were lysed by sonication in a lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5% (v/v) NP-40] containing protease inhibitor cocktail (Sigma-Aldrich Japan) and phosphatase inhibitor cocktail (Sigma-Aldrich Japan). Protein concentrations in the tissue lysates were determined using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific Japan). The lysates (40 μg of protein) and a molecular weight standard (MagicMark XP; Thermo Fisher Scientific Japan) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, on a 15% gel) under reducing conditions and transferred to a polyvinylidene difluoride membrane. After blocking with Tris-buffered saline (pH 7.4) containing 5% skim milk and 0.1% (v/v) Tween-20 for 1 h, the membrane was incubated overnight at 4°C with any of the two rabbit polyclonal anti-SR-B1 antibodies (1 : 3000, NB400-104; Novus Biologicals, Littleton, CO, USA, and TA301489; OriGene, Rockville MD, USA). After several washes with Tris-buffered saline (pH 7.4) containing 0.1% (v/v) Tween-20, a horseradish peroxidase-labelled polyclonal swine anti-rabbit IgG secondary antibody (1 : 3000, DAKO P0399; Agilent, Santa Clara, CA, USA) was added. Immunolabelled protein complexes were detected using an ImageQuant LAS-4000 mini chemiluminescent imager (GE Healthcare Japan, Tokyo). For probing CD36, a goat polyclonal anti-CD36 antibody (1 : 2000, AF2519; R&D Systems, Minnesota, MN, USA) and peroxidase-labelled polyclonal rabbit anti-goat IgG secondary antibody (1 : 1000, DAKO P0449; Agilent) were used as primary and secondary antibodies, respectively.

**Immunohistochemistry.** The mice were anaesthetised with sodium pentobarbital (10 mg/kg body weight) and were perfused via the aorta with 0.9% (w/v) saline (0.5 mL/g) followed by a fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). The heads were removed and immersed in the same fixative for an additional 2 days. For decalcification, the heads were immersed in 5% ethylenediaminetetraacetic acid for 3 weeks at 4°C. The decalcified heads were immersed overnight at 4°C in 30% sucrose solution, embedded in optimal cutting temperature compound (Sakura Finetek, Tokyo, Japan) and quickly frozen in liquid nitrogen. Frozen liver sections were prepared as described previously (19). Frozen sections from the mice and livers of the mice were mounted on poly-L-lysine-coated glass slides. After pre-treatment with PBS containing 0.3% (v/v) Triton X-100 (PBST) and normal donkey serum, the sections were incubated overnight with TA301489 (1 : 200). After several washes with PBST, sections were incubated with DAKO P0399 (1 : 160) for 60 min. The slides were then washed with PBS, and colour was developed using a Histofine™ immunostaining kit (Nichirei Co., Tokyo, Japan). The stained sections were counterstained with haematoxylin. When the sections were incubated with NB400-104 as a primary antibody, Cy3-labelled donkey polyclonal anti-goat IgG (1 : 200, 705-165-147; Jackson ImmunoResearch, West Grove, PA, USA) was used as the secondary antibody, and the nuclei were counterstained with SYTO 13 (Thermo Fisher Scientific). The stained sections were mounted in glycerol/PBS and observed under a confocal laser-scanning microscope (FV1000; Olympus, Tokyo, Japan). For double immunofluorescence staining, the sections were incubated overnight with TA301489 and AF2519. They were then incubated with Alexa Fluor® 488-labelled donkey anti-rabbit IgG (1 : 200; Thermo Fisher Scientific Japan) and Cy3-labelled donkey anti-goat IgG. The stained sections were observed as described above.

**RESULTS**

Design and development of b5S-SRB1_{187–206} a peptide mimic of SR-B1

To date, there have been no reports demonstrating the ability of SR-B1 to recognise odour-active volatile compounds in the environment. To this end, we devised an assay system to characterise SR-B1 ligands. We previously developed a cell-free in-vitro assay for the identification of CD36 ligands (29–33). In the assay, we used a solid support onto which certain synthetic peptides containing the oxLDL binding site of CD36 (Fig. 1A) were immobilised (e.g., one containing amino acid residues 149–168 of the receptor [hereafter mentioned as CD36 (149–168)], designated b5S-CD36_{149–168} in this study, Fig. 1B). This system allowed the estimation of CD36 ligand activity in test compounds by measuring their ability to inhibit fl-oxLDL binding to the support (30–35). In this study, we devised a similar cell-free assay system for screening of SR-B1 ligands.

A shorter amino acid segment of SR-B1 (183–205) fused to glutathione S-transferase was previously found to bind smaller unilamellar vesicles containing oxGPC_{SRB} (see Fig. 1A) (9), suggesting that the region represents the site for oxLDL binding. In this study, we chose to devise and use a synthetic peptide consisting of residues 187–206 from
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A
SR-B1 (187–206)  
LVNLIN YFPGMF PKDKFG  
Φ ⧢ Φ ζΦΦΦ ΦΦ *ζ*
CD36 (149–168)  
YQNQF VQMI LNSL INKSSS

B

\( \text{b5S-SRB1}_{187-206} \) (scramble)  
\( \text{Biotin-} \text{SSSSSLVNLIK YFPGMF PKDKFG} \)

\( \text{b5S-SRB1}_{187-206} (3K/N) \)  
\( \text{Biotin-} \text{SSSSSLVNLIK YFPGMF PKFNDNFG} \)

\( \text{b5S-CD36}_{149-168} \)  
\( \text{Biotin-} \text{SSSSSYQNQF VQMI LNSL INKSSS} \)

Fig. 1 Design of a peptide mimic of SR-B1. (A) Alignment of SR-B1 (187–206) and CD36 (149–168). In both receptors, the sequence of the human counterpart is shown in a single-letter code. Bold and thin asterisks indicate Lys and Asn residues that are identical in alignment, respectively. Hydrophobic and hydrophilic residues to be aligned are indicated by Φ and ζ, respectively. (B) Amino acid sequences of peptides used. The sequences of b5S-SRB1_{187-206}, b5S-SRB1_{187-206} (scramble), b5S-SRB1_{187-206} (3K/N), and b5S-CD36_{149-168}, any of which are biotinylated at their N-termini, are shown in a single-letter code. Residues substituted with Asn in b5S-SRB1_{187-206} (3K/N) are indicated by asterisks. Ser residues added to the N-terminal side of each peptide are underlined.

SR-B1 [hereafter mentioned as SR-B1 (187–206)] as a mimic of the receptor for binding oxLDL (b5S-SRB1_{187-206}, Fig. 1B). This was primarily based on alignment of Lys202 and Lys204 in SR-B1 with Lys164 and Lys166 in CD36, which were demonstrated to be essential for binding of oxLDL/oxGPCSRB in CD36 (Fig. 1A) (14, 29–31). The peptide chain length (20 amino acids) was also aligned with CD36 (149–168), by referring to the utility of b5S-CD36_{149-168} to bind fl-oxLDL. We synthesised two additional variants of b5S-SRB1_{187-206}: one with a scrambled amino acid sequence, designated b5S-SRB1_{187-206} (scramble), and another in which Lys residues at positions 193, 202 and 206 of SR-B1 were substituted with Asn residues, designated b5S-SRB1_{187-206} (3K/N) (Fig. 1B). Note that (i) each of the SR-B1 peptides, like b5S-CD36_{149-168} (35), has five additional Ser residues on its N-terminal side in the expectation that these residues would contribute in improving the solubility of the peptide, and (ii) the peptides are N-terminally biotinylated for immobilization onto solid supports (streptavidin-precoated plates) via avidin-biotin interaction.

Utility assessment of b5S-SRB1_{187-206} as an artificial SR-B1
We examined whether b5S-SRB1_{187-206} could be used to detect fl-oxLDL binding. The mean fluorescence value of wells pre-treated with b5S-SRB1_{187-206} was higher than that of untreated control wells (Fig. 2A). The mean fluorescence of wells pre-treated with b5S-SRB1_{187-206} was lower in those containing non-labelled oxLDL than in those that did not contain non-labelled oxLDL. These results indicate the specific binding interaction between b5S-SRB1_{187-206} and fl-oxLDL. This is the first evidence indicating that a short segment of SR-B1 binds oxLDL.

No apparent differences in the mean fluorescence value were observed between wells pre-treated with b5S-SRB1_{187-206} (scramble) and untreated control wells (Fig. 2A). In the wells that were pre-treated with b5S-SRB1_{187-206} (scramble), fluorescence readings were similar, irrespective of the presence or absence of non-labelled oxLDL (Fig. 2A). The results obtained in wells pre-treated with b5S-SRB1_{187-206} (3K/N) were similar to those obtained in the wells pre-treated with b5S-SRB1_{187-206} (scramble) (Fig. 2A). These data indicate that the amino acid sequence of b5S-SRB1_{187-206} is critical for specific binding interaction with fl-oxLDL and that positively charged residues in the SR-B1 segment, such as those at positions 164 and 166 of CD36 (14, 29–31), play essential roles in recognising oxLDL. Notably, in SR-B1, Lys residues at positions 202 and 204 are
conserved in several mammalian species, whereas that at position 193 is not (Fig. S1). It seems likely that the conserved Lys residues play major roles in oxLDL recognition.

The specific binding of fl-oxLDL to plates that were pre-treated with b5S-SRB1_{187-206} exhibited a dose-dependent saturation pattern (Fig. 2B). By fitting the progress curve of the amount of fl-oxLDL bound to a well of the plate versus the concentration of fl-oxLDL in the well to the theoretical curve using equation 1, the $K_d$ and $B_{\text{max}}$ values were determined to be 3.3 μg protein/mL and 28 ng protein/well, respectively. Gillotte-Taylor and co-workers previously determined the $K_d$ value for the binding of iodine-125-labelled oxLDL to full-length SR-B1 expressed by a mammalian cultured cell line (4.0 μg protein/mL) (11). It seems unlikely that the binding affinity of oxLDL for b5S-SRB1_{187-206} differs to a large degree from that for the full-length receptor. Therefore, we postulate that only a short segment in SR-B1 is sufficient for specific binding of oxLDL, similar to CD36.

The binding of oxLDL to SR-B1-expressing cultured mammalian cells was previously found to be inhibited by oxGPC SRB species, but poorly or not at all inhibited by non-oxidised GPC species (3, 9). We investigated whether KOdiA-PC and DPPC as representatives of oxGPC SRB and non-oxidised GPC species, respectively, could inhibit fl-oxLDL–b5S-SRB1_{187-206} binding. Wells pre-treated with b5S-SRB1_{187-206} were incubated with fl-oxLDL in the absence and presence of various concentrations of GPC species. KOdiA-PC inhibited binding in a concentration-dependent manner with an IC_{50} value of 89 μM ($R^2 = 0.97$). On the other hand, DPPC exhibited little ability to inhibit fl-oxLDL binding at the concentrations tested. The IC_{50} value determined for the non-oxidised GPC was more than 10 mM ($R^2 = 0.26$). This suggested that b5S-SRB1_{187-206} has ligand specificity similar to that of full-length SR-B1. The results shown in Fig. 2 validate the utility of b5S-SRB1_{187-206} as a peptide mimic of SR-B1 to evaluate ligand activities of test substances.

Evaluation of SR-B1 ligand activities of volatile odorants present in the environment by the assay utilising b5S-SRB1_{187-206}

We have recently shown that odorants with an aldehyde moiety, including saturated aliphatic aldehydes with 9 to 16 carbon atoms (e.g., tetradecanal) and unsaturated ones (e.g., Z-11-hexadecenal), exhibit inhibitory activity against fl-oxLDL binding to peptide mimics of CD36 (32, 33, 35). On the other

![Fig. 2](image-url)
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were consistent with those obtained previously (35), i.e. decanal, dodecanal, tetradecanal, hexadecanal, and E-2-tridecenal exhibited inhibitory activities, whereas hexanal, octanal, octadecanal, benzaldehyde, and citral did not (Fig. 3). In this study, we showed the inhibitory activity of Z-11-hexadecenal upon using b5S-SRB1187–206 (Fig. 3) for the first time. In view of the inhibitory effect of individual aldehydes, the results obtained using b5S-SRB1187–206 were comparable to those obtained using the CD36 peptide (Fig. 3). The difference was that citral showed an inhibitory activity with the use of the SR-B1 mimic (22% inhibition) (Fig. 3). Based on these results, we conclude that SR-B1 (187–206) can recognise certain volatile odorants as potential ligands.

We selected five aliphatic aldehydes that displayed more than 50% inhibition of fl-oxLDL binding to b5S-SRB1187–206 at 2 mM (Fig. 3), which were: decanal, dodecanal, tetradecanal, hexadecanal, and Z-11-hexadecenal; and determined their IC_{50} values for inhibition. Among these, tetradecanal had the most potent activity (IC_{50} of 0.67 mM) (Table 1). The IC_{50} values for aldehydes other than tetradecanal were more than 1 mM (Table 1). For comparison, we determined the IC_{50} values for these aldehydes in the use of b5S-CD36_{149–168} under the same conditions. Notably, the IC_{50} values obtained for decanal and dodecanal in the use of b5S-SRB1187–206 were comparable to those obtained using the CD36 peptide (Fig. 3). The difference was that citral showed an inhibitory activity with the use of the SR-B1 mimic (22% inhibition) (Fig. 3). Based on these results, we conclude that SR-B1 (187–206) can recognise certain volatile odorants as potential ligands.

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similar to those in the use of b5S-CD36_149–168, but the values for tetradecanal, hexadecanal and Z-11-hexadecenal (in particular for Z-11-hexadecenal) were higher in using the SR-B1 mimic than in using the CD36 mimic (Table 1). These results indicate that the efficiencies of the aliphatic aldehydes binding with 14 and 16 carbon atoms differ between b5S-SRB1_187–206 and b5S-CD36_149–168. As representatives, the inhibition curves for decanal, tetradecanal and Z-11-hexadecenal are illustrated in Fig. 4.

**Table 1** Assessment and comparison of inhibitory activities of aliphatic aldehydes against fl-oxLDL binding to b5S-SRB1_187–206 and b5S-CD36_149–168

<table>
<thead>
<tr>
<th>Aliphatic aldehydes</th>
<th>b5S-SRB1_187–206</th>
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<tbody>
<tr>
<td></td>
<td>IC_{50} (mM)</td>
<td>R^2</td>
</tr>
<tr>
<td>Decanal</td>
<td>1.2 (1.1 to 1.3)</td>
<td>0.95</td>
</tr>
<tr>
<td>Dodecanal</td>
<td>1.3 (1.1 to 1.6)</td>
<td>0.86</td>
</tr>
<tr>
<td>Tetradecanal</td>
<td>0.67 (0.58 to 0.78)</td>
<td>0.94</td>
</tr>
<tr>
<td>Hexadecanal</td>
<td>1.8 (1.4 to 2.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Z-11-Hexadecenal</td>
<td>1.1 (0.87 to 1.5)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

All assays were performed using two to three independent wells. The values for IC_{50} (and the 95% confidence interval) and the coefficient of determination (R^2) were calculated using Prism® software.

RT-PCR and western immunoblot analyses of SR-B1 expression in the nasal tissue of mice

To obtain evidence for the existence of SR-B1 in the nasal tissue, we performed RT-PCR analysis to evaluate the mRNA expression of SR-B1 quantitatively. An amplicon of the expected size for SR-B1 mRNA (706 bp) was detected in a nasal sample (Fig. 5A, left panel). This assay detected a 706-bp band from a liver sample (a reference tissue for SR-B1 mRNA expression) (2) as well as from the nasal sample (Fig. 5A, upper right panel). Note that the band density in the nasal sample was lower than that in the liver sample.

Next, we analysed SR-B1 protein expression in the nasal mucosa and liver of mice by SDS-PAGE and western blotting. An anti-SR-B1 antibody (NB400-104) produced a dense band of the expected size for SR-B1 protein (80 kDa) in the liver sample (Fig. 5B, left panel). An 80-kDa band was also detected in the sample of nasal mucosa, although the signal intensity was lower than that in the liver sample (Fig. 5B, left panel). We also conducted this analysis using an additional anti-SR-B1 antibody (TA301489). The antibody detected an 80-kDa protein band in the tissue sample as well as the liver sample (Fig. S2A). We analysed the level of CD36 protein in the nasal and liver samples using an antibody directed against the receptor (AF2519) as a probe. The level of the 88-kDa band (indicative of CD36 protein) was similar between the two tissue samples (Fig. 5B, right panel), this being consistent with our previous report (17). Together with the data for RT-PCR analysis, we concluded that SR-B1 is expressed in the nasal tissues of mice, albeit at low levels.

**Immunolocalisation of SR-B1 protein in the olfactory mucosa of mice**

We finally analysed the localisation of SR-B1 protein in the nasal mucosa by immunohistochemistry. To assess the utility of the anti-SR-B1 antibody TA301489 for immunolocalisation, we first stained liver sections. Immunoreactivity was found mostly along the outline of cavities, and was thought to arise from sinusoidal endothelial cells (Fig. 6A) (8). The signals were less evident on the cell surface of hepatic parenchymal cells (Fig. 6A). The pattern of staining was comparable to that obtained by Ganesan and co-workers using anti-SR-B1 antibodies other than TA301489 (8). Based on these results, we concluded that antibody TA301489 could satisfactorily demonstrate receptor localisation.

When sections of the nasal mucosa were probed with antibody TA301489, the surface layer (i.e., the mucus layer) of the olfactory epithelium was stained positively (Fig. 6B, C). Weak staining was also observed beneath the layer (i.e., in the somata of olfactory supporting cells) (Fig. 6B, C). On the other hand, little to no staining was detected from the deeper epithelial layer where olfactory sensory neurons are located (Fig. 6B, C) and in the lamina propria (Fig. 6B). Nasal respiratory mucosa, including the epithelium, also showed little or no staining (Fig. 6B). A similar staining pattern for nasal mucosa was found with an anti-SR-B1 antibody NB400-104, except that the staining of the somata in olfactory supporting cells was less evident (Fig. S2B).
A novel role for SR-B1

When we performed double immunostaining of olfactory epithelium using anti-SR-B1 and anti-CD36 antibodies (TA301489 and AF2519, respectively), both antibodies yielded dense signals in the surface layer while a population of olfactory sensory neurons were immunolabelled only by the CD36 antibody (Fig. 7). The merged image revealed that immunoreactive SR-B1 was less intense in the most superficial layer (i.e., ciliary layer) (also refer to Fig. 6B).

DISCUSSION

In this study, we provided evidence that SR-B1 recognises certain aliphatic aldehydes as potential ligands, and it occurs in the surface layer of the olfactory epithelium in mice. Based on the results presented here, we propose a novel role for SR-B1 as a contributor to the capture of specific volatile odorants in the nasal cavity of mammals. One important question is whether the inhibitory activities of test compounds expressed as IC₅₀ determined by our in-vitro assay using physiological buffered saline are indicative of their interaction with SR-B1 on the surface of olfactory epithelium. We previously determined the IC₅₀ value for an aliphatic aldehyde, oleic aldehyde (C18:1), against fl-oxLDL binding to mimic peptides for CD36 to be about 1 mM under conditions similar to that employed in this study (32, 33, 35). It was noted that a small amount of oleic aldehyde spotted on filter paper (i.e., the aldehyde in gas phase) was perceived in mice through a mechanism involving CD36 (18). Our interpretation of these results is that the binding affinities between CD36 and odorants are underestimated in physiological saline. In other words, the receptor-dependent recognition of volatile compounds on the olfactory epithelial surface is much more effective than expected. Indeed, the olfactory mucus layer is known to provide an environment for efficient binding between odorants and their binding proteins (7). We expect that the aliphatic aldehydes listed in Table 1 are at least recognisable by class B scavenger receptors, including SR-B1, in the nasal cavity of mammals. However, it remains uncertain if these aliphatic aldehydes directly bind to the peptide mimics of class B scavenger receptors to compete against fl-oxLDL binding. We cannot rule out the possibility that these aldehydes at high micromolar concentrations merely deform fl-oxLDL to inhibit the binding to peptides. Assays (e.g., surface plasmon resonance assay) to characterise the direct binding of aliphatic aldehydes to peptide mimics should be developed.

SR-B1 (187–206) was thought to have a slightly lower ability to recognise aliphatic aldehydes with 14 or 16 carbon atoms compared to CD36 (149–
For instance, the IC\textsubscript{50} value for Z-11-hexadecenal was determined to be about 1.6-fold higher in the use of b5S-SRB\textsubscript{187–206} than that in the use of b5S-CD\textsubscript{36}\textsubscript{149–168} (Table 1 and Fig. 4). It can be assumed that CD36 (149–168) has amino acid residues that assist in interaction with such aliphatic compounds, whereas SR-B1 (187–206) does not (Fig. 1A). Based on the lack of CD36 ligand activity of aliphatic aldehydes with a shorter hydrocarbon chain (e.g., hexanal and octanal), we postulated the importance of hydrophobic interactions between the hydrocarbon chain moiety of aliphatic aldehydes and the hydrophobic residues of the receptor in their binding (35). Note that in CD36, the residue at position 154 is a bulky hydrophobic one across several mammalian species, whereas SR-B1 has a polar non-charged residue at the aligned position (position 191) (Fig. S1). It is tempting to speculate that (i) the bulky hydrophobic residue in CD36 (149–168) participates in the recognition of aliphatic aldehydes with 14 and 16 carbon atoms, and (ii) the lower ability of b5S-SRB\textsubscript{187–206} to bind such aliphatic

**Fig. 5** RT-PCR and western immunoblot analyses for assessment of SR-B1 expression in the nasal tissue of mice. (A) RT-PCR analysis. A representative result for RT-PCR detection of SR-B1 mRNA from a nasal sample is presented in the left panel (lane 1). A DNA size marker (100 bp DNA Ladder, New England Biolabs Japan, Tokyo) was loaded in lane M. The positions at which 100-, 500-, and 1000-bp fragments migrate are indicated on the left in base pairs (bp). Right panels show representative data for RT-PCR detection of SR-B1 (upper) and β-actin (lower) mRNAs in the nasal mucosa (Nasal) and liver of mice. (B) Western immunoblot analysis. Lysates obtained from the nasal mucosa (Nasal) and liver of mice were analysed by SDS-PAGE and western blotting with an anti-SR-B1 antibody (NB400-104) (left panel). In the right panel, a western blot with an anti-CD36 antibody (AF2519) for nasal mucosa and liver samples is shown. Samples were analysed in duplicate for either of the receptors. In either panel, the positions where marker proteins migrate are indicated on the left in kilo Daltons (kDa).
Fig. 6 Immunolocalisation of SR-B1 in the nasal mucosa. Sections were probed with an anti-SR-B1 antibody (TA301489). (A) Immunostaining of the liver. Immunoreactive SR-B1 was found along the outline of cavities, which was thought to arise from sinusoidal endothelial cells. (B) Immunostaining of sections containing both the nasal olfactory and respiratory mucosa. The boundary between the nasal olfactory and respiratory mucosa is indicated by an arrowhead. Immunoreactive SR-B1 was found in the surface layer of olfactory epithelium (OE) but not in that of respiratory epithelium (RE). (C) A high magnification image for the staining of the olfactory epithelium. Bar: 20 μm (A, C), 50 μm (B).

Fig. 7 Double immunostaining of the olfactory epithelium in mice with an anti-CD36 antibody (AF2519) (top panel) and an anti-SR-B1 antibody (TA301489) (middle panel). The merged image is shown in the bottom panel. Within the image, ten or more CD36-immunopositive olfactory sensory neurons in the deeper epithelial layer are illustrated (red). Of these, the ones in which the slender process being extended to the apical surface (one of the histologic signatures of olfactory sensory neurons) is identifiable, are indicated by arrowheads. Bar in the top panel: 20 μm.

compounds is attributed to the lack of hydrophobic residues at position 191. It is also noted that the IC_{50} values for decanal and dodecanal (C10 and C12, respectively) obtained using b5S-SRB1_187-206 were similar to those obtained using b5S-CD36_149-168. An explanation is that the hydrophobic residues aligned at positions 194 to 200 in SR-B1 and 156 to 162 in CD36 (Fig. 1A and Fig. S1) are sufficient for hydrophobic interaction in the recognition of these aliphatic aldehydes.

CD36 is produced by a population of olfactory sensory neurons, and is transported to and accumulates in the cilia, the first line for olfactory percep-
tion of odorants (17, 22, 38). The most likely role of the receptor at this site has been postulated to serve as an obligate transmembrane partner for G-protein-coupled odorant receptors, facilitating the recognition of specific odorants (4, 12, 38). In contrast to CD36, there was no apparent evidence for the existence of SR-B1 in the somata of olfactory sensory neurons (Fig. 6A, B and Fig. 7). Furthermore, immunoreactive SR-B1 was hardly detected in the most superficial layer of olfactory epithelium (Fig. 7). Therefore, it seems unlikely that SR-B1 locates on the olfactory cilia and plays a role similar to CD36 in olfaction. Immunohistochemical analysis with an anti-SR-B1 antibody TA301489 implied the expression and production of SR-B1 in olfactory supporting cells (mucus-producing cells), which project apical microvilli (Fig. 6A, B and Fig. 7). If this is true, we predict that immunoreactivity to SR-B1 in the surface layer of the olfactory epithelium should arise from the receptor localised on the microvilli. Certain sensory neuron membrane proteins (SNMPs), the insect homologues of mammalian class B scavenger receptors, are located in supporting cells around the olfactory sensory neurons in organisms (4, 25). CD36 is also known to be present on or around the microvilli of olfactory supporting cells (17, 38). As for SNMPs, SNMP-2 (in Heliothis virescens) is assumed to participate in quick pheromone clearance of the cuticular sensilla lymph to allow highly sensitive olfactory detection (6). By analogy, one of the possible roles of olfactory SR-B1 is to clean up the mucus layer of the olfactory epithelium via trapping odorants. However, because of the close proximity between microvilli and cilia in the mucus layer (10), we cannot exclude the possibility that SR-B1 gains access to and transfers odorants to olfactory receptors (i.e., it participates in the perception of odorants). Further studies are required to define the precise role and site of action of SR-B1 in the olfactory epithelium. Regardless, our present findings on SR-B1 coupled with the recent findings on CD36, contribute to expanding our knowledge of the function of class B scavenger receptors.

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Fig. S2  Further confirmation of the existence of SR-B1 protein in the nasal tissue of mice. (A) Western immunoblot analysis of lysates from the nasal mucosa (Nasal) and liver of mice using an anti-SR-B1 antibody (TA301489). The 80-kDa band representing SR-B1 protein is indicated by an arrowhead on the right of the panel. Samples were analysed in duplicate. The positions at which the marker proteins (Precision Plus Protein™ unstained Standards, Bio-Rad, Hercules, CA, USA) migrate are indicated on the left in kilo Daltons (kDa). (B) Immunostaining of a section containing both the nasal olfactory and respiratory mucosa with an anti-SR-B1 antibody (NB400-104). OE, olfactory epithelium and RE, respiratory epithelium. Bar: 50 μm.