Neuroscience 142 (2006) 1263–1271

COMPLETE OVERLAP OF INTERLEUKIN-31 RECEPTOR A AND ONCOSTATIN M RECEPTOR β IN THE ADULT DORSAL ROOT GANGLIA WITH DISTINCT DEVELOPMENTAL EXPRESSION PATTERNS

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Abstract—Interleukin-31 receptor A (IL-31RA) is a newly identified type I cytokine receptor, that is related to gp130, the common receptor of the interleukin (IL) -6 family cytokines. Recent studies have shown that IL-31RA forms a functional receptor complex for IL-31 together with the β subunit of oncostatin M receptor (OSMRβ). However, little is known about the target cells of IL-31 because it remains unclear which types of cells express IL-31RA. In our previous reports, we demonstrated that OSMRβ is expressed in a subset of small-sized nociceptive neurons of adult dorsal root ganglia (DRGs). In the present study, we investigated the IL-31RA expression in the adult and developing DRGs. From a northern blot analysis and *in situ* hybridization histochemistry, IL-31RA mRNA was found to be expressed in the adult DRGs. According to reverse-transcriptase polymerase chain reaction, IL-31RA mRNA was detected in the DRGs and trigeminal ganglia, while no expression of IL-31RA mRNA was observed in the CNS. Double immunofluorescence staining revealed IL-31RA to be expressed in a subset of small-sized neurons, all of which colocalized with OSMRβ. In addition, the expression of IL-31 RA was detected in afferent fibers in the spinal cord and the dermis of the skin. We also found that the developmental expression pattern of IL-31RA was different from that of OSMRβ; IL31RA-positive neurons in DRGs first appeared at postnatal day (PN) 10 and reached the adult level at PN14, whereas OSMRβ-positive neurons were observed at PN0 for the first time.

We previously demonstrated OSMRβ-expressing neurons to decrease, however, they were not found to disappear in oncostatin M (OSM) -deficient mice. These findings suggest that IL-31 and OSM may thus have redundant functions in the development of OSMRβ-expressing neurons.

Key words: interleukin-31, oncostatin M, receptor, dorsal root ganglion, mouse.

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Abbreviations: DIG, digoxigenin; DRG, dorsal root ganglion; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; IL-31RA, interleukin-31 receptor A; ir, immunoreactivity; OSM, oncostatin M; OSMRβ, β subunit of oncostatin M receptor; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline with 0.1% Triton X-100; PCR, polymerase chain reaction; PFA, paraformaldehyde; PI3K, phosphoinositide-3-kinase; PN, postnatal day; RT, room temperature; RT-PCR, reverse transcriptase–polymerase chain reaction; SSC, saline-sodium citrate buffer; STAT, signal transducers and activators of transcription; TG, trigeminal ganglion; TRPV1, transient receptor potential vanilloid 1.

Oncostatin M (OSM) belongs to the interleukin (IL) -6 family of cytokines, which share the common signaling receptor subunit, gp130, in their multimeric receptors (Kishimoto et al., 1994). Mouse OSM activates the specific receptor composed of the β subunit of oncostatin M receptor (OSMRβ) and gp130 (Tanaka et al., 1999). In contrast, human OSM activates two types of receptor complex, OSMRβ/gp130 heterodimer and leukemia inhibitory factor receptor β/gp130 heterodimer (Ichihara et al., 1997). Thus, experimental systems using mouse OSM have revealed unique biological activities mediated through the OSMRβ in the definitive hematopoiesis (Mukouyama et al., 1998; Miyajima et al., 2000) and the differentiation of hepatocytes (Kamiya et al., 1999; Miyajima et al., 2000).

In our previous studies, we demonstrated that OSMRβ is expressed in a subset of small-sized nonpeptidergic nociceptive neurons in dorsal root ganglia (DRGs), and all OSMRβ-expressing neurons expressed both transient receptor potential vanilloid 1 (TRPV1) and P2X3 purinergic receptor (Tamura et al., 2003). In addition, we also reported that OSM-deficient mice exhibit a reduced number of OSMRβ-expressing DRG neurons as well as reduced responses to the noxious stimuli (Morikawa et al., 2004; Morikawa, 2005). Therefore, OSMRβ-expressing DRG neurons may function as nociceptors. In fact, in a model of inflammatory hyperalgesia induced by the injection of complete Freund's adjuvant (CFA) to the plantar skin, OSM was found to be produced by the inflammatory cells infiltrating to the dermis and the phosphorylation of both signal transducers and activators of transcription (STAT) -3 and cyclic AMP-responsive element binding protein is up-regulated in OSMRβ-expressing DRG neurons (Tamura et al., 2005).

Interleukin-31 receptor A (IL-31RA), also known as gp130-like monocyte receptor or GP130-like receptor, is a recently identified type I cytokine receptor, which displays similarities with gp130 (Dillon et al., 2004; Diveu et al., 2003, 2004; Dreuw et al., 2004; Ghilardi et al., 2002). IL-31RA acts as a functional receptor complex for IL-31, together with OSMRβ (Dillon et al., 2004). Through the receptor complex, IL-31 induces the activation of various signal molecules, including STAT proteins and mitogen-activated protein kinase (MAPK) (Diveu et al., 2004).

IL-31 has recently been reported to induce dermatitis in mice (Dillon et al., 2004). The expression of IL-31 has been shown to increase in the skin of patients with atopic dermatitis (Bilsborough et al., 2006; Sonkoly et al., 2006). Although IL-31 might be related to the pathogenesis of the dermatitis, the target cells for IL-31 remain to be eluci-

0306-4522/06$30.00+0.00
doi:10.1016/j.neuroscience.2006.07.009

dated. In the present study, we investigated the IL-31RA expression in the DRGs using *in situ* hybridization and immunohistochemical methods.

EXPERIMENTAL PROCEDURES

Mice

In the present study, postnatal (0-, 7-, 10-, 12-, and 14-day-old) and adult (6- to 10-week-old) C57BL/6J male mice (Clea Japan, Tokyo, Japan) were used. These mice were kept under a 12-h light/dark cycle with food and water. At all times, the experiments were carried out under the control of the Animal Research Control Committee in accordance with the Guidelines for Animal experiments of Wakayama Medical University, Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23) revised 1978. All efforts were made to minimize the number of animals used and their suffering.

Northern blot analysis

A northern blot analysis was performed with some modifications as previously described (Bando et al., 2005). Briefly, total RNA was isolated by Tri Reagent (Molecular Research Center, Cincinnati, OH, USA) from the dissected regional brain, trigeminal ganglia (TGs), and DRGs (L4/L5) of adult mice according to the manufacturer's protocol. Total RNA (20 μg) was fractionated in a 1.2% agarose gel containing 2.4% formaldehyde and transferred to a positively charged nylon membrane (Roche Diagnostics, Indianapolis, IN, USA). The blotted membrane was hybridized with a digoxigenin (DIG) -labeled single-stranded cDNA probe for IL-31RA in hybridization buffer (50% formaldehyde, 5× saline–sodium citrate buffer [SSC; 33.3 mM sodium chloride, 33.3 mM trisodium citrate, pH 7.0], 0.05 M phosphate buffer, pH 7.0, 7% sodium dodecyl sulfate, 2% blocking reagent, 0.1% lauroylsarcosine) at 42 °C for 16 h. After washing the membrane to remove the nonspecifically bound probe, the blotted membrane was incubated with alkaline phosphatase-conjugated anti-DIG antibody (Roche Diagnostics) at room temperature (RT) for 2 h. Labeled probes were detected with chemiluminescence using CDP-Star (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. Thereafter, the membrane was exposed to X-ray film for an appropriate period.

Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis

Total RNA was prepared from postnatal and adult DRGs using Tri Reagent and then was reverse-transcribed using the first strand cDNA synthesis kit (Amersham Biosciences) with random primers. The following oligonucleotide primers specific to mouse IL-31RA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used: for IL-31RA, 5′-CAGAGAACAGCACTAACCTCA-3′ (sense) and 5′-AATCTCCTTCCATGTGATCGTG-3′ (antisense); for GAPDH, 5′-ACCACAGTCCATGCCATCAC-3′ (sense) and 5′-TCCACCACCCTGTTGCTGTA-3′ (antisense). Polymerase chain reaction (PCR) was done at 95 °C for 2 min for the initial denaturing, followed by 30 cycles at 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 60 s. The amplified PCR products were electrophoresed on 1.2% agarose gel and then were visualized with ethidium bromide.

Preparation of IL-31RA probes for both the Northern blot analysis and *in situ* hybridization histochemistry

The mouse IL-31RA cDNA probes were obtained by PCR amplification from a mouse testis cDNA library. Briefly, a 521 bp fragment (coding region 854-1374) of IL-31RA was amplified using the IL-31RA-specific primers described. The PCR product was ligated into a pGEM-T easy vector (Promega, Madison, WI, USA). In northern blotting, a DIG-labeled single-stranded DNA probe for IL-31RA was made by PCR by using the IL-31RA-specific antisense primer described above, dNTP, and DIG-11-dUTP (Roche Diagnostics). For *in situ* hybridization histochemistry, the plasmid containing the fragment of mouse IL-31RA cDNA was linearized by cutting with *Nco*I (for sense probe) or with *Spe*I (for anti-sense probe). *In vitro* transcription was performed using appropriate RNA polymerase (SP6 RNA polymerase for the sense probe and T7 RNA polymerase for the antisense probe) and [α-^{35}S] UTP.

In situ hybridization histochemistry

In situ hybridization histochemistry was performed as described previously (Morikawa et al., 2004). Briefly, adult mice were anesthetized with diethyl ether and transcardially perfused with ice-cold 0.85% NaCl, followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS). The DRGs (L4/L5) were quickly taken out, post-fixed with 4% PFA in PBS at 4 °C for 4 h, and then immersed in 30% sucrose in PBS. They were embedded in O.C.T medium (Miles, Elkhart, IN, USA), frozen rapidly in cold *n*-hexane on dry ice, and stored at −80 °C.

Frozen sections of DRGs were cut on a cryostat at 6-μm thickness. After treatment with Proteinase K (Roche Diagnostics), the sections were postfixed with 4% PFA in 0.1 M PBS, treated with acetic anhydride, and then were dehydrated with graded ethanol. The sections were hybridized with ^{35}S-labeled sense or antisense cRNA probe at 55 °C for 16 h. After rinsing in 2× SSC containing 10 mM dithiothreitol, the sections were treated with ribonuclease A (20 mg/ml; Wako Pure Chemical Industries, Tokyo, Japan). High stringency washes were performed in 0.1× SSC at 55 °C. After dehydration, the slides were submerged in NTB-2 liquid emulsion (Kodak, Rochester, NY, USA). After 14 days of exposure, the dipped autoradiograms were developed in D-19 developer (Kodak) and fixed. The sections were counterstained through the emulsion with hematoxylin, and examined under bright- and dark-field microscopy (XF-WFL, Nikon, Tokyo, Japan).

Immunohistochemistry

Immunostaining was performed with some modifications as previously described (Tamura et al., 2003). Postnatal and adult mice were anesthetized by diethyl ether and transcardially perfused with 0.85% NaCl, followed by ice-cold modified Zamboni's fixative (2% PFA and 0.2% picric acid in 0.1 M PBS, pH 7.4). DRGs (L4/L5), spinal cord, and plantar skin were quickly taken out, post-fixed with modified Zamboni's fixative at 4 °C for 3 h, and then immersed in 20% sucrose in PBS. They were embedded in O.C.T. medium, frozen rapidly in cold *n*-hexane on dry ice and stored at −80 °C. Slide-mounted 6-μm cryostat tissue sections of DRGs (L4/L5) and 20-μm sections of spinal cord and plantar skin were processed for immunostaining.

The sections were preincubated in phosphate-buffered saline with 0.1% Triton X-100 (PBS-T) containing 5% normal donkey serum at RT for 1 h, and then incubated with primary antibodies in PBS-T containing 1% bovine serum albumin (4 °C, overnight). Primary antibodies were used at the following dilutions: goat anti-IL-31RA 1:100 (R&D Systems, Minneapolis, MN, USA); rat anti-OSMRβ 1:100 (MBL, Nagoya, Japan); rabbit anti-gp130 1:500 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were washed in PBS-T and were incubated in Cy2- or Cy3-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) diluted 1:400 with PBS-T containing 1% BSA (RT, 1 h). Immunofluorescent images were acquired using a Nikon VFM microscope with fluorescent attachment.

In situ hybridization combined with immunohistochemistry

As described previously (Tamura et al., 2004), immunohistochemistry was performed following *in situ* hybridization. Briefly, the sections were hybridized with IL-31RA cRNA probe, and then were used for immunohistochemistry immediately after the final wash. They were preincubated in 0.1 M PBS containing 5% normal goat serum at RT for 1 h, and then incubated with goat anti-IL-31RA antibody (1:100, R&D Systems) at 4 °C overnight. After washes in PBS, the sections were incubated with biotinylated donkey anti-goat immunoglobulin G antibody (1:800, Jackson ImmunoResearch) and an avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, Germany). The reaction was visualized with 0.05% diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries) and 0.01% H_2O_2. After the reaction, they were submerged in the liquid emulsion.

Quantitative analysis

A quantitative analysis was performed as described previously (Tamura et al., 2003). Briefly, L4/L5 DRGs were taken from five adult mice perfused with Zamboni's fixative, pooled, and processed for sectioning. All ganglia were sectioned at 6 μm. For each DRGs, five sections that were 120 μm apart were selected for immunostaining. Only neurons with clearly visible nuclei and nucleoli were counted. To visualize all neuronal profiles, the sections were incubated with NeuroTrace (Molecular Probes, Eugene, OR, USA) at 1:500 dilution for 20 min and 4′,6-diamidino-2-phenylindole dihydrochloride for 5 min for nuclear staining after incubation with secondary antisera. Photographic images were obtained using an epifluorescent Nikon Eclipse E800 microscope. Positive neurons were counted and cell sizes were measured using the NIH image analysis software program (Scion, Frederick, MD, USA).

RESULTS

Expression of IL-31RA mRNA in CNS and peripheral nervous tissue specimens

We first examined the expression of IL-31RA mRNA in various regions of the CNS, TGs, and DRGs using a northern blot analysis. As shown in Fig. 1A, a single band of 4.8 kb corresponding to IL-31RA mRNA was strongly expressed in DRGs, while IL-31RA mRNA was not detected in TGs and other brain regions, including the cortex, cerebellum, olfactory bulb, hypothalamus, hippocampus and spinal cord. In addition, an RT-PCR analysis revealed that a single band corresponding to IL-31RA mRNA was detected in TGs and DRGs, while no bands were observed in any other of the examined brain regions (Fig. 1B).

Identification and distribution of IL-31RA-expressing neurons in the DRGs

As IL-31RA mRNA was detected in adult DRGs by a northern blot analysis, we performed *in situ* hybridization histochemistry to identify the type of cells expressing IL-31RA mRNA. The IL-31RA-expressing cells were clearly observed in a subpopulation of DRG neurons, not in satellite cells (Fig. 2A). The sense cRNA probe failed to hybridize in the DRGs (Fig. 2B). To check the specificity of the anti-IL-31RA antibody we used, *in situ* hybridization combined with immunohistochemistry was performed. As

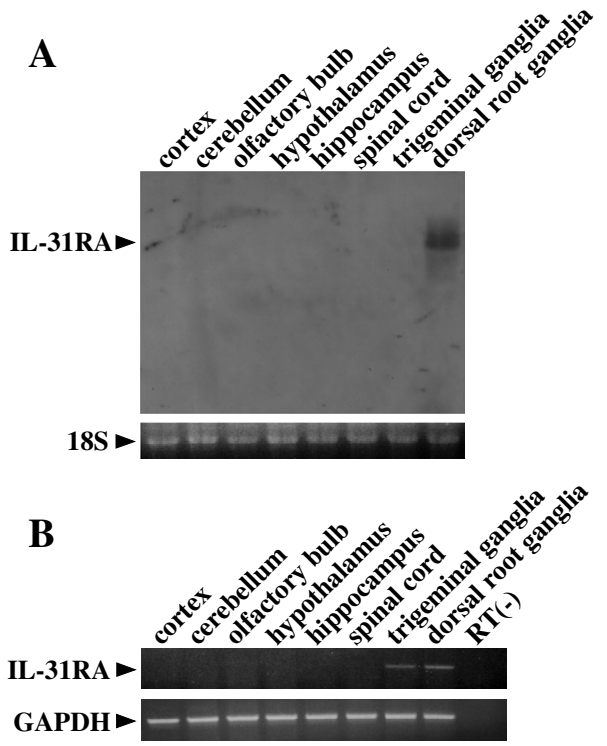

Fig. 1. Distribution of IL-31RA mRNA in the CNS and peripheral nervous system of adult mice. (A) The upper panel shows that IL-31RA mRNA was detected in the DRG using a northern blot analysis. Equal loading of total RNA was checked by 18S ribosomal RNA visualized by ethidium bromide (lower panel). (B) RT-PCR analysis revealed IL-31RA mRNA in the TGs in addition to the DRG (upper panel). GAPDH was used as an internal control (lower panel).

shown in Fig. 2C, all the IL-31RA mRNA-expressing cells were IL-31RA-immunoreactive, confirming the specificity of the anti-IL-31RA antibody.

We next quantified the expression of IL-31RA in DRG neurons by immunofluorescence staining on representative sections of L4/L5 DRGs. Strong immunofluorescence was observed in a subpopulation of small-sized DRG neurons in their cytoplasm. In these ganglia, 12.6±1.3% (254 of 2016 cells) of total DRG neurons contained IL-31RA and the expression was most prevalent in small-sized neurons (range 99–347 μm^2), as indicated by the peak of the histogram (150–200 μm^2) (Fig. 2D).

Colocalization of IL-31RA and OSMRβ in the DRG neurons

As IL-31RA and OSMRβ form a functional receptor complex for IL-31 (Dillon et al., 2004), we examined the colocalization of IL-31RA with OSMRβ in the DRG neurons. Double-immunofluorescence staining for IL-31RA and OSMRβ in adult DRG neurons revealed that immunofluorescence for OSMRβ completely overlapped with that for IL-31RA (Fig. 3A–F), thus suggesting that functional receptor complex for IL-31 can be formed in a subset of small-sized DRG neurons.

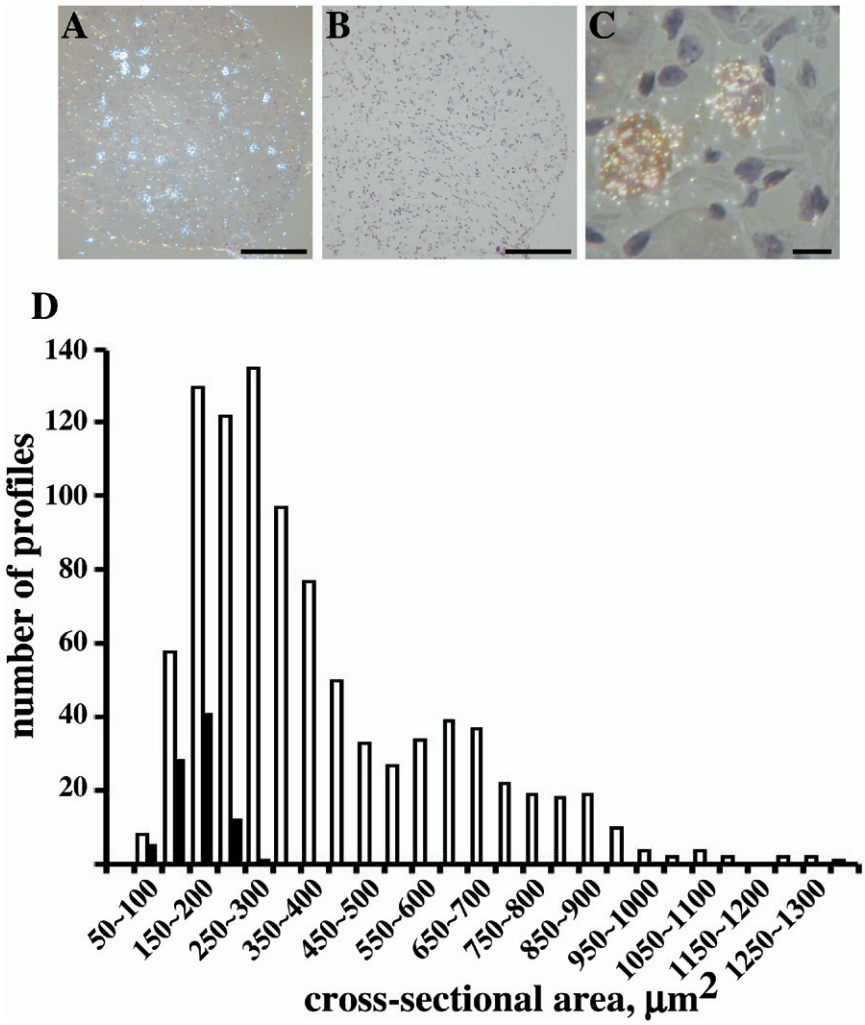

Fig. 2. (A) *In situ* hybridization histochemistry for IL-31RA mRNA in the adult DRGs. (B) The corresponding sense riboprobe failed to hybridize in the DRGs. (C) Immunohistochemistry of IL-31RA combined with *in situ* hybridization. IL-31RA protein (brown) was detected in DRG neurons containing IL-31RA mRNA (white dots). Scale bars=200 μm (A, B), 40 μm (C). (D) A size frequency histogram for IL-31RA-positive neurons. The distribution of the cross-sectional area of IL-31RA-positive neuronal profiles (black bar) and all neuronal profiles (white bar) was investigated in L4/5 DRGs from four adult mice. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Expression of IL-31RA in both central and peripheral processes of DRG neurons

Primary afferent fibers containing OSMRβ-immunoreactivity (ir) were limited within the inner portion of lamina II in the dorsal horn of spinal cord. In addition, OSMRβ-expressing nerve fibers were also observed in the dermis of the skin (Tamura et al., 2003). Double-immunofluorescence staining for IL-31RA and OSMRβ in the spinal cord and dermis revealed that IL-31RA was colocalized with OSMRβ in both the primary afferent fibers projecting to the inner portion of lamina II in the dorsal horn of spinal cord (Fig. 4A–C) and the dermis (Fig. 4D–F).

Expression of IL-31RA and OSMRβ in the developing DRGs

As our previous study demonstrated the expression of OSMRβ mRNA in the DRGs to first be detected at postnatal day (PN) 0 while thereafter reaching the adult level at PN 14 (Morikawa et al., 2004), we investigated the developmental expression pattern of IL-31RA. An RT-PCR analysis showed the expression of IL-31RA mRNA to first be weakly detected at PN10 and its level increased gradually as the development proceeded (Fig. 5A). We next performed double-immunofluorescence staining for IL-31RA and OSMRβ in the developing DRGs. At PN7, no IL-31RA-ir was detected, while OSMRβ protein was detectable in a subset of neurons (Fig. 5B–D). At PN10, IL-31RA-ir was first detected in quite a small number of neurons, which were colicalized with OSMRβ (Fig. 5E–G). At PN12, IL-31RA-ir was clearly detected in the OSMRβ-expressing cells, and the ratio of IL-31RA-positive cells in OSMRβ-positive neurons increased (Fig. 5H–J). At PN14, IL-31RA-ir was detected in all the OSMRβ-positive neurons, thus indicating that the expression level of IL-31RA reached the level of adult DRGs (Fig. 5K–M). At each stage, IL-31RA-ir was exclusively detected in the OSMRβ-positive neurons.

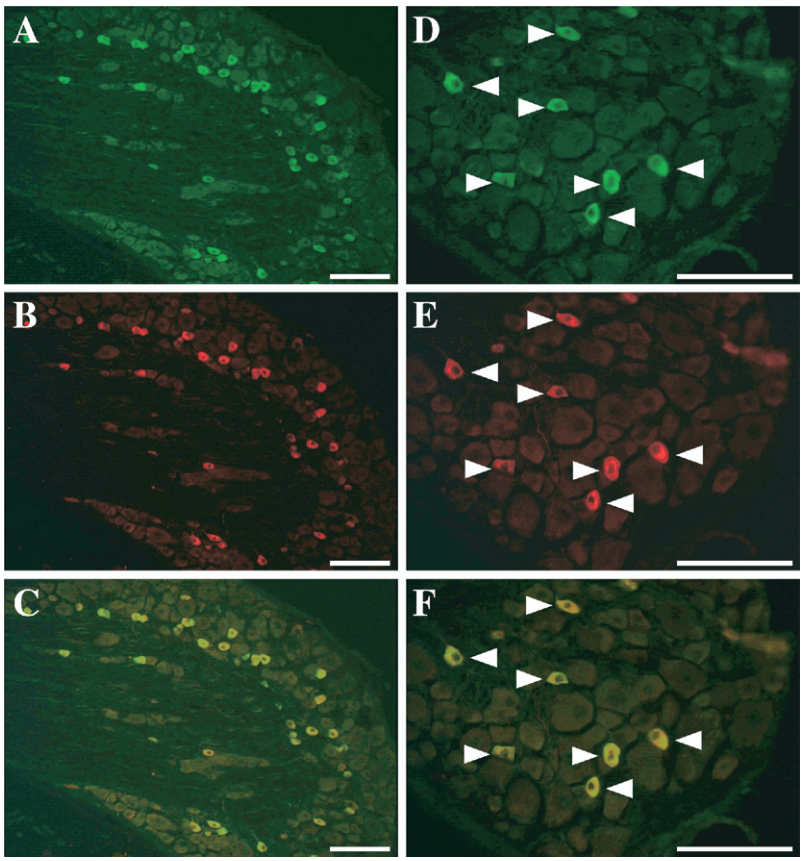

Fig. 3. Double-immunofluorescence staining of IL-31RA and OSMRβ in the adult DRGs. Immunofluorescence images show IL-31RA (A and D, green) and OSMRβ (B and E, red). All the neurons with IL-31RA-ir contained OSMRβ (C and F, yellow). IL-31RA/OSMRβ-double positive cells are indicated by arrowheads (D–F). Panels D, E, and F are high-power view of panels A, B, and C, respectively. Scale bars=200 μm (A–C); 100 μm (D–F).

Expression of gp130 and OSMRβ in the developing DRGs

We next investigated the expression of gp130 in the developing DRGs because gp130 forms a functional receptor complex for OSM together with OSMRβ. At PN0, gp130-ir was detected in many DRG neurons (Fig. 6B), a few of which express OSMRβ (Fig. 6A and C). Consistent with our previous study (Morikawa et al., 2004), OSMRβ-expressing neurons increased as the development proceeded, and reached the adult level at PN14 (Fig. A, D, G). Throughout postnatal stages, all the OSMRβ-expressing neurons also contained gp130-ir at PN7 (Fig. 6D–F) and at PN14 (Fig. 6G–I).

Expression of IL-31RA in neurons of TGs

The expression of IL-31RA was observed in small-sized sensory neurons of the adult TGs as well as the DRGs

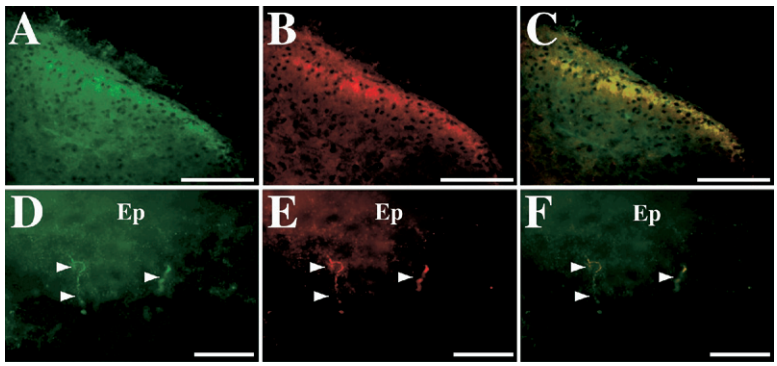

Fig. 4. Double-immunofluorescence staining of IL-31RA and OSMRβ in adult dorsal horn (A–C) and plantar skin (D–F). Immunofluorescence images show IL-31RA (A, and D, green) and OSMRβ (B and E, red) and merged image (C and E). Note that IL-31-positive fibers co-expressed OSMRβ in the lamina II of dorsal horn of spinal cord. IL-31-positive fibers co-expressing OSMRβ were also detected in the dermis of the skin (arrowheads). Scale bars=200 μm (A–C); 25 μm (D–F).

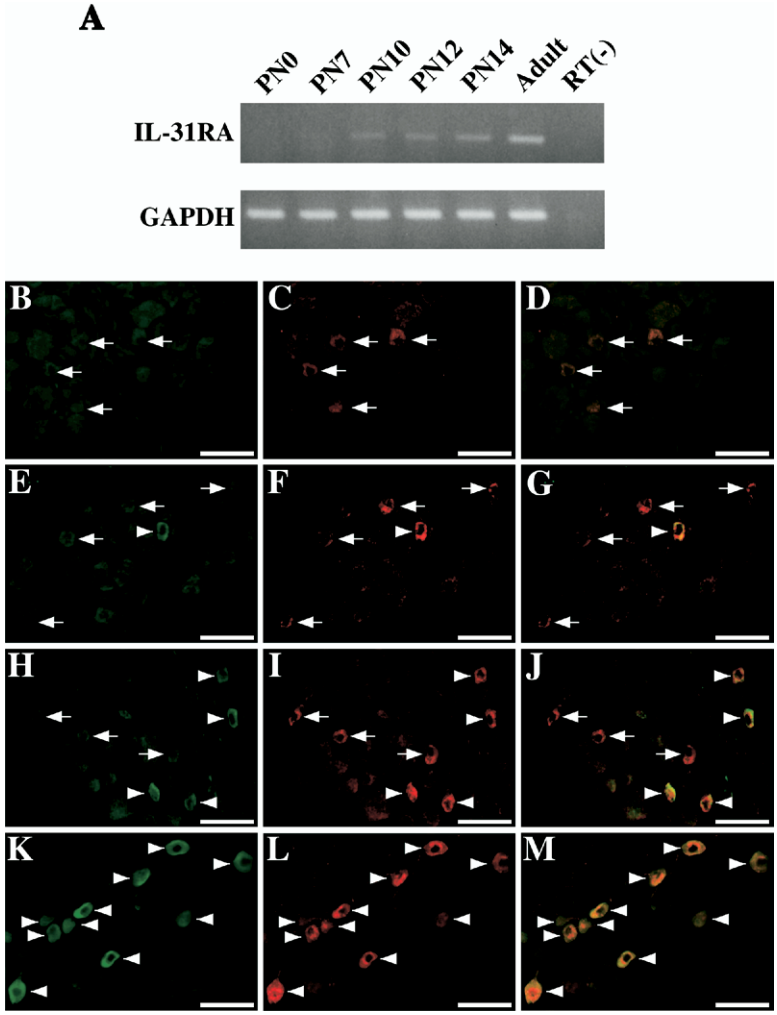

Fig. 5. Expression of IL-31RA in the developing DRGs. (A) Expression of IL-31RA mRNA was assessed by RT-PCR analysis in DRG at various developmental stages. Upper and lower panels showed expression of IL-31RA mRNA and GAPDH mRNA, respectively. PCR conditions were described in Experimental Procedures. (B–M) Double-immunofluorescence staining for IL-31RA (B, E, H and K, green) and OSMRβ (C, F, I and L, red) at various postnatal stages, PN7 (B–D), PN10 (E–G), PN12 (H–J) and PN14 (K–M). The cells of colocalization appear yellow in the merged images (D, G, J and M). Arrowheads and arrows indicate IL-31RA/OSMRβ-double positive cells and OSMRβ-single positive cells, respectively. Scale bars=50 μm.

(Fig. 7). IL-31RA-positive neurons in the TGs were less numerous than those in the DRGs. This result is compatible with the amounts of mRNA revealed by both northern blot and RT-PCR analyses (Fig. 1). Double-immunofluorescence staining revealed that all the IL-31RA-positive neurons in the TGs were labeled with anti-OSMRβ antibody (Fig. 7). These results are therefore consistent with those in the DRGs.

DISCUSSION

In the present study, we found that IL-31RA mRNA was highly expressed in the adult DRGs by both northern blot and RT-PCR analyses (Figs. 1 and 5A). *In situ* hybridization histochemistry revealed that IL-31RA mRNA was expressed in a subset of small-sized DRG neurons. In addition, all the IL-31RA-expressing neurons contained OSMRβ in adult DRGs. These results suggest that a functional receptor for IL-31 exists in a subset of small-sized DRG neurons, which are TRPV1/P2X3/OSMRβ-triple positive (Tamura et al., 2003). In addition, our previous study has demonstrated that OSMRβ-expressing neurons also expressed gp130 in the adult DRGs (Tamura et al., 2003). Taken together, a subset of small-sized OSMRβ-expressing DRG neurons could be signaled from both IL-31 and OSM. Recently, the intracellular signaling mechanism of IL-31 has been reported to be a little different from that of OSM. Both IL-31 and OSM could activate STAT-1, -3 and -5, ERK1/2, Akt and SHP-2. On the other hand, phosphoinositide-3-kinase (PI3K), which is strongly phosphorylated by OSM, could be activated marginally by IL-31, even though a high dose of IL-31 was applied (Diveu et al., 2004). These observations suggest that OSM may have distinct physiological functions from those of IL-31 in a subset of small-sized DRG neurons through the activation of PI3K.

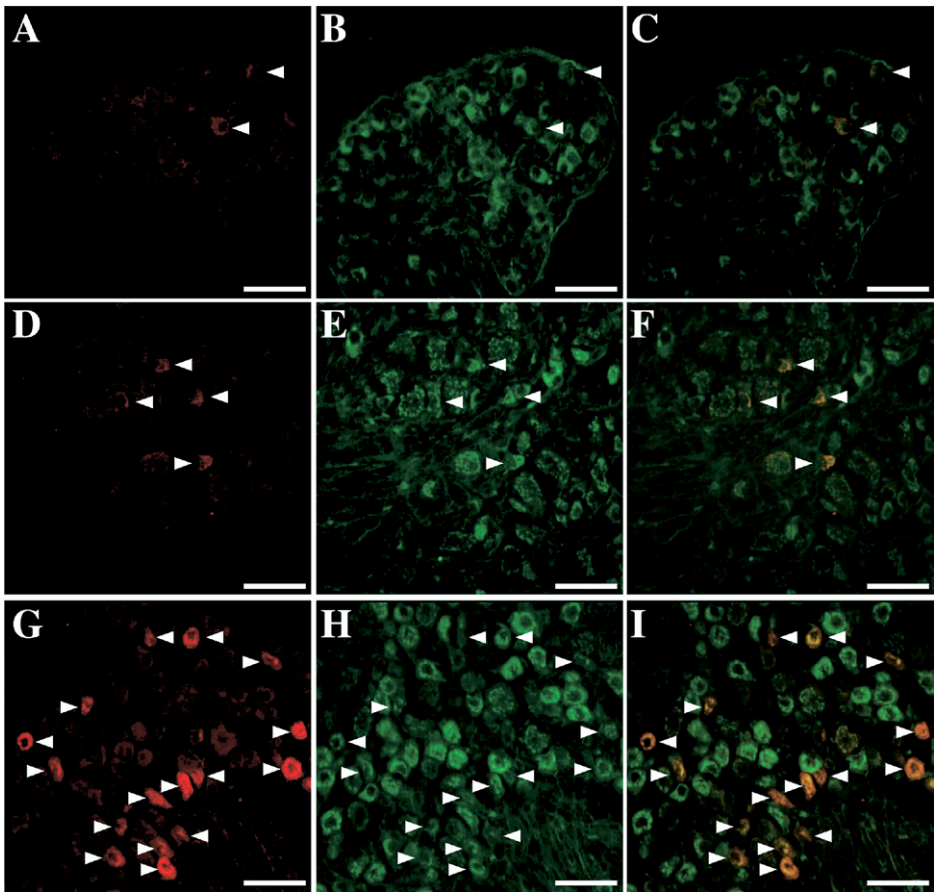

Fig. 6. Double-immunofluorescence staining for gp130 and OSMRβ in the developing DRG neurons. These sections were obtained from mice at PN0 (A–C), PN7 (D–F) and PN14 (G–I). Immunofluorescence images for OSMRβ (A, D and G, red) and gp130 (B, E and H, green) were merged, and gp130/OSMRβ-double positive cells appear yellow in the merged images (C, F and I). Arrowheads indicate gp130/OSMRβ-double positive cells. Scale bars=50 μm.

We previously reported that all the OSMRβ-expressing DRG neurons express both TRPV1 and P2X3 (Tamura et al., 2003), which are activated by proton and ATP, respectively (Chen et al., 1995; Lewis et al., 1995; Caterina et al., 1997). The fact that a specific subset of DRG neurons expressed a functional receptor complex may lead to the involvement of IL-31 signaling in the nociceptive function. On the other hand, IL-31 is expressed in the skin lesion of human patients with atopic dermatitis, the main symptom of which is itch (Bilsborough et al., 2006; Sonkoly et al., 2006). Likewise, NC/Nga mice, a mouse model that develops atopic dermatitis, shows the increase in IL-31 in the skin lesion and itch-associated scratch behavior (Takaoka et al., 2005, 2006). In addition, IL-31-overexpressing mice tend to develop skin lesions similar to those seen in atopic dermatitis (Dillon et al., 2004). In the present study, IL-31RA was colocalized with OSMRβ in the nerve fibers of a specific subset of DRG neurons in the dermis, thus suggesting that IL-31 produced in the skin could exert its effects on nerve fibers and terminals. Although the relationship of itching with pain remains unclear, IL-31RA/OSMRβ-double positive neurons may be responsible for itching as well as pain. In fact, histamine induces itching through H1 receptors, which are expressed in isolectin B4-positive, non-peptidergic, small-sized DRG neurons (Kashiba et al., 1999). Such natures of DRG neurons responsible for histamine-induced itch are similar to those of IL-31RA/OSMRβ-double positive neurons.

OSMRβ forms receptor complexes with gp130 and IL-31RA, which function as receptors for OSM and IL-31, respectively. During the postnatal development of DRGs, OSMRβ-positive neurons first appeared at PN0, gradually increase, while reaching the adult level at PN14 (Morikawa et al., 2004). The expression of IL-31RA was first detected in DRGs at PN10 and its level at PN14 was the same as that observed in the adulthood. Throughout the postnatal period, as far as we could determine, all the IL-31RA-poisitive neurons contained OSMRβ. In contrast, the expression of gp130 was detected in many neurons of the DRGs from PN0 to PN14, and all the OSMRβ-positive neurons expressed gp130, as shown in Fig. 6. These findings suggest that the functional receptor complex for IL-31 can be formed in a subset of DRG neurons from PN10, whereas that for OSM can be expressed in a subset of DRG neurons from PN0. Our previous study revealed that the number of OSMRβ-expressing DRG neurons significantly decreased, but they did not disappear, in OSM-deficient mice (Morikawa et al., 2004), thus suggesting that

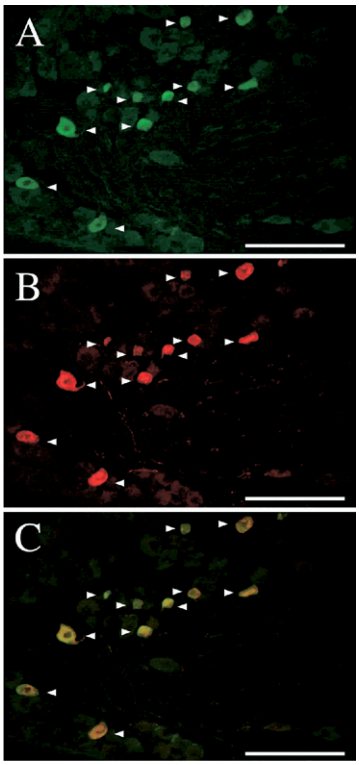

Fig. 7. Expression of IL-31RA in TG neurons of adult mice. IL-31RA-ir was also observed in small-sized neurons in the adult TG (A, green). Double-immunofluorescence staining indicated that all the IL-31RA-expressing neurons (A, green) were positive for OSMRβ (B, red). The double-positive cells (A–C, arrowheads) appear yellow in the merged image (C). Scale bars=100 μm.

other factors may compensate in part for OSM in the development of DRGs. Because IL-31RA and gp130 genes are located in close genomic loci and show a very similar exon/intron structure (Diveu et al., 2003), it has been considered that IL-31RA and gp130 genes might thus have been evolved by gene duplication event (Diveu et al., 2003). It is therefore hypothesized that IL-31 and OSM may thus have redundant functions in the development of DRGs, while the expression pattern of their functional receptor complexes is different in the developmental period.

Acknowledgments—This work was supported by a Research Grant on Priority Areas from Wakayama Medical University.

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(Accepted 11 July 2006)
(Available online 22 August 2006)