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(54) Title: ANTI ONCOSTATIN M RECEPTOR BETA ANTIBODY

(57) Abstract: A monoclonal antibody against oncostatin M specific receptor beta subunit, a hybridoma capable of producing the same and a medicament for treating atopic dermatitis comprising the same.

[Kind of Document] Description

[Title of the Invention] ANTI ONCOSTATIN M RECEPTOR BETA ANTIBODY

[Technical Field]

[0001]

The present invention relates to an antibody against oncostatin M (OSM) receptor beta subunit (OSMRβ), a hybridoma capable of producing the monoclonal antibody and use of the monoclonal antibody for the treatment of OSM receptor-related diseases or disorders such like atopic dermatitis.

[Background Art]

[0002]

The immune system protects human body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. However, the immune response can sometimes be unwanted and cause immune-mediated disorder. The disorder includes autoimmune disease, graft rejection, hypersensitivity, diseases associated with the over-stimulation of host's immune system by microbes. The autoimmune diseases result from immune responses against endogenous and/or exogenous antigens. Foreign substances, derived from bacteria, parasites, fungi or viruses, may mimic self-proteins and cause the immune system to erroneously launch an immune attack on self-cells and tissues, resulting in onset of the autoimmune diseases. The graft rejection is caused by the immune response in the transplant recipient (host) against the transplanted organ/tissue. When a subject is transplanted with grafts including kidney, pancreas, heart, lung, bone marrow, cornea and skin, the subject can launch an immune response (rejection) against the grafts. Hypersensitivity is an inappropriate immune response that has deleterious effects, resulting in significant tissue damage or even death. The response is characterized by the overproduction of cytokines. The exaggerated production of cytokines is known to contribute to sepsis characterized by cytokine-mediated lethal shock (Espat NJ, et al. J Surg Res. 1995 Jul; 59 (1):153-8). Multiple organ dysfunction syndromes (MODS) are a major cause of morbidity and mortality in severe sepsis and shock. Cytokine-mediated lethal shock resulted from over-production of host cytokines is considered a main mechanism leading to MODS (Wang H, et al. Am J Emerg Med. 2008 Jul; 26 (6):711-5).

[0003]

Atopic dermatitis is a pruritic inflammatory skin disease. The conventional medicinal agent for treating the atopic dermatitis is mainly a topical cream comprising as an active ingredient steroid compounds. However, such available medical agents are not always effective in the most critical symptoms such like pruritus in addition of exhibiting serious side effects caused by steroid

compounds per se. Thus, the alternative agent possessing potent effect against pruritus and no serious side effect is expected to be provided.

[0004]

The demonstrated in vivo activities of the cytokine family illustrate the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and cytokine antagonists. Recently, several investigators have reported that interleukin (IL)-31 is involved in the pathogenesis of atopic dermatitis. Functional IL-31 receptor consists of IL-31 specific receptor A (IL-31Ra) and OSMRβ.

[0005]

Oncostatin M (OSM) is a member of the IL-6 family of cytokines and its receptor consists of the OSM specific receptor beta subunit (OSMRβ) and gp130, the common receptor subunit of this cytokine family. OSM is produced by a variety of cells such as hematopoietic cells and fibroblasts and is suggested to play a role in immune reactions and hematopoiesis, while both OSM-deficient mice and OSMRβ-deficient mice developed normally and were fertile (Tanaka et al., 2003).

[0006]

Interleukin-31 is produced by activated $CD4^+$ T cells. Overexpression of IL-31 in transgenic mice results in a pruritic skin condition that is similar to human atopic dermatitis (Dillon et al., 2004). Analysis of IL-31 levels in human dermatitis samples has shown increased expression of IL-31 in atopic dermatitis compared with healthy control populations, implicating involvement of IL-31 in the pathogenesis of allergic skin diseases (Bilsborough et al., 2006; Sonkoly et al., 2006). IL-31 receptor alpha (IL-31Ra) pairs with OSMRβ to form the functional heterodimer receptor for IL-31 (Dillon et al., 2004). In addition, the present inventors have demonstrated complete co-localization of IL-31Ra and OSMRβ in both a subset of small-sized dorsal root ganglion (DRG) neurons and afferent fibers in the spinal cord and the dermis of the skin (Bando et al, 2006). Thus, IL-31 might be related to the pathogenesis of the dermatitis. However, treatment of NC/Nga mice with severe atopic dermatitis with anti-IL-31 antibody failed to ameliorate skin lesions in spite of the reduction of scratching behavior (Grimstad et al., 2008), suggesting that blocking of IL-31 is not enough to prevent atopic dermatitis development.

[0007]

Nucleic acid and amino acid sequences of OSMRβ are known and sequenced (SEQ. ID Nos.1 and 2, respectively), and OSMRβ is suggested to be associated with the biological activities mediated by OSM (WO95/33059).

[0008]

It is known that an antagonist of OSM such as an antibody thereto or a small molecule can be used for the treatment or prophylaxis of an inflammatory arthropathy or inflammatory disorder, and for screening for such antagonists (WO99/48523).

[0009]

5 One of the present inventors studied actions of OSM on a pain-responsive neuron and provided a pharmaceutical composition for treating pains, especially treatment-resistant pain such as cancerous pain, neurogenic pain and inflammatory pains, which contains an OSM antagonist or the OSM or contains a transgenic vector containing a nucleic acid in which a cytotoxic gene is linked to a promoter of an OSM receptor beta-chain gene (JP 2005-247836 A).

10 [0010]

In the previous report, the present inventors have demonstrated that OSMRβ and IL-31Ra were co-expressed in the same subset of small-sized nociceptive neurons of adult dorsal root ganglia (DRGs).

[Disclosure of Invention]

15 [0011]

The present inventions relate to an antibody against oncostatin M (OSM) receptor beta subunit (OSMRβ), a hybridoma capable of producing the monoclonal antibody and use of the monoclonal antibody for the treatment of OSM receptor-related diseases or disorders such like atopic dermatitis.

20 [Problem to be solved by the Invention]

[0012]

The present invention addresses these needs by providing the antibody to pro-inflammatory cytokine receptor, namely OSM receptor (OSMR). Such antibody of the present invention, which may block, inhibit, reduce antagonize or neutralize the activity of OSM. The invention

25 further provides uses therefor in inflammatory disease, as well as related compositions and methods.

[Means for Solving the Problem]

[0013]

To examine a role of OSM and IL-31 during atopic dermatitis development, the present inventors

30 eagerly studied OSMRβ and antibodies against the same and developed anti-OSMRβ specific monoclonal antibody 7D2, and then evaluated the function of the antibody in atopic dermatitis mice.

[0014]

The present inventors investigated the function of the signaling from OSMRβ with itch and/or

skin lesions of atopic dermatitis. In OSMRβ-deficient mice, IL-31-induced itch responses were abolished, indicating that IL-31 evokes itch through OSMRβ. Then, the present inventors developed anti-OSMRβ specific antibody for further animal studies. The present inventors evaluated the function of the antibody in NC/Nga mice, which developed skin lesions that were
5 similar to human atopic dermatitis. The subcutaneous application of anti-OSMRβ antibody decreased scratching behavior with dramatic amelioration of the skin lesions in the NC/Nga mice. In addition, the elevation of total serum IgE was suppressed and serum IL-13 level was decreased in the anti-OSMRβ antibody-treated mice.

[0015]

10 These findings indicate that the OSMRβ is provided as a potential molecular target for therapeutic intervention and anti-OSMRβ specific antibody is effective for immunotherapy in patients with atopic dermatitis. Based on these findings, the present inventors have completed the invention.

[0016]

15 Thus, the present inventions are as follows:

(1) A hybridoma cell line deposited as Accession No. FERM ABP-11380.

(2) A monoclonal antibody against oncostatin M specific receptor beta subunit, obtained from the hybridoma according to (1).

(3) An active fragment of the antibody according to (2), selected from the group
20 consisting of a Fab, Fab', $F(ab')_2$, and scFv.

[0017]

(4) An inhibitor against the signal pathways between OSM and OSM receptor and between IL-31 and IL-31 receptor, comprising the monoclonal antibody according to (2) or the fragment according to (3).

25 [0018]

(5) A medicament for treating atopic dermatitis comprising the monoclonal antibody according to (2) or the fragment according to (3).

(6) Use of the monoclonal antibody according to (2) or the fragment according to (3) for manufacturing the medicament for treating atopic dermatitis.

30 (7) A method for treating atopic dermatitis comprising a therapeutically effective amount of the monoclonal antibody according to (2) or the fragment according to (3) with a pharmaceutically acceptable carrier, excipient or diluent.

[0019]

(8) A method for isolation and identification of a molecule possessing more potent

binding activity against OSMRβ as well as against OSM receptor consisting of OSMRβ and gp130 in comparison with the monoclonal antibody according to (2), comprising the following steps:

1) preparing and incubating said molecule with (a) the cells expressing OSMRβ and expressing no gp130, and with (b) the cells expressing OSM receptor, in the presence of the monoclonal antibody according to (2); and

2) indirectly investigating whether said molecule can competitively bind to both of said cells by measuring the binding of the monoclonal antibody according to (2) to both of said cells.

(9) A method for isolation and identification of a molecule possessing more potent inhibition activities against OSM and/or IL-31 signal pathways in comparison with the monoclonal antibody according to (2), comprising the following steps:

1) preparing and incubating said molecule with the cells expressing OSM receptor and IL-31 receptor; and

2) investigating whether said molecule can more significantly suppress the phosphorylation activity induced by signal transduction from OSM to OSM receptor and/or from IL-31 to IL-31 receptor in comparison with the monoclonal antibody according to (2).

[Brief Description of the Drawings]

[0020]

FIGs. 1A and 1B show induction of scratching behavior by IL-31 in mice. FIG. 1A shows scratching behavior induced by IL-31 in C57BL/6J mice (n = 4 per groups). Mice were
5 injected PBS (white bar), IL-31 (25-1000 ng; black bars), or OSM (400 ng; gray bar) in the rostral part of the back skin intradermally. FIG. 1B shows scratching behavior induced by IL-31 in OSMRβ$^{+/+}$ and OSMRβ$^{-/-}$ mice (n = 4 per groups). OSMRβ$^{+/+}$ (white bars) and OSMRβ$^{-/-}$ (black bars) mice were injected PBS or IL-31 (400 ng) in the rostral part of the back skin intradermally. Scratching behavior was counted for 2 hours. Data represent the means +
10 SEM. $*P < 0.05$ versus PBS-injected mice in A. $*P < 0.05$ versus PBS-injected OSMRβ$^{+/+}$ mice in B. $\#P < 0.05$ versus IL-31-injected OSMRβ$^{+/+}$ mice in B, Student's *t*-test.

[0021]

FIGs. 2A-2D show generation of anti-OSMRβ antibody (7D2). FIG. 2A shows that Ba/F3-mOSMRβ transfectants were stained with 7D2 hybridoma supernatant. Isotype control
15 staining; gray shaded, 7D2 staining; bold line. FIG. 2B shows isotyping test of 7D2 monoclonal antibody. Isotype of 7D2 monoclonal antibody was determined as rat IgG1 kappa. FIG. 2C shows purification of 7D2 monoclonal antibody. Purity of 7D2 monoclonal antibody was evaluated with SDS-PAGE followed by CBB staining. 1 μg of purified 7D2 monoclonal

antibody was loaded. FIG. 2D shows that Ba/F3-mOSMRβ and Ba/F3-mIL31Ra transfectants were stained with anti-OSMRβ (7D2) or anti-IL31Ra antibody (antibody concentration: 5 μg / ml). Note that 7D2 monoclonal antibody was specifically stained for BaF3-mOSMRβ but not for BaF3-mIL31Ra.

[0022]

FIGs. 3A and 3B show characterization of 7D2 monoclonal antibody. FIG. 3A shows that LO cells express OSMRβ and gp130. The cells were stained with anti-OSMR antibodies. Antibody 30-1 was purchased from MBL. Both 7D2 and 30-1 monoclonal antibodies stained LO cells while 7D2monoclonal antibody had better staining ability for Ba/F3-mOSMRβ than for 30-1 (antibody concentration: 5 μg / ml). FIG. 3B shows affinity evaluation of anti-OSMR antibodies. Ba/F3-mOSMRβ or LO cells were stained with several concentration of the antibodies 7D2 and 30-1. After flow cytometric analysis, the percentage of stained cells (% of positive cells) was plotted with the antibody concentrations. X axis: antibody concentration (μg / ml), Y axis: percentage of the stained cells at the antibody concentration.

[0023]

FIGs 3C and 3D show the results of western blot analysis for Extracellular Signal-regulated Kinases (Erk). Upper and lower arrows indicate phosphorylated Erk1 and Erk2, respectively. Phosphorylated Erk1 and Erk2 were detected by using the specific antibody against phosphorylated Erk1 and Erk2 (i.e., anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody). FIG. 3C shows that stimulation of OSM or IL-31 can induce the phosphorylation of Erks. FIG. 3D indicates that 7D2 monoclonal antibody can suppress the Erk phosphorylation caused by stimulation of OSM or IL-31, suggesting that 7D2 monoclonal antibody can inhibit the signal pathways of OSM/OSM receptor and IL-31/IL-31 receptor.

[0024]

FIG. 4 shows effects of an anti-OSMRβ antibody on scratching behavior of NC/Nga mice. NC/Nga mice were implanted with the hydrogel incorporated with anti-OSMRβ 7D2 monoclonal antibody (dotted line: n = 7) or its isotype control antibody (solid line: n = 6) at two times (day 0 and day 14). Scratching behavior was counted for 2 hours on days 0, 14, 24, and 31. Data represent the means ± SEM. ANOVA followed by the post hoc Bonferroni test.

[0025]

FIG. 5 shows effects of an anti-OSMRβ antibody on macroscopic observations of skin lesions in NC/Nga mice. NC/Nga mice were implanted with a hydrogel incorporated with anti-OSMRβ 7D2 monoclonal antibody (D-F) or its isotype control antibody (A-C) at two times (day 0 and day 14). Representative photographs on day 0 (A and D), day 14 (B and E), and day 31 (C and

F) were shown.

[0026]

FIG. 6 shows effects of an anti-OSMRβ antibody on dermatitis of NC/Nga mice. NC/Nga mice were implanted with a hydrogel incorporated with anti-OSMRβ 7D2 monoclonal antibody (dotted lines; n = 8) or its isotype control (solid lines: n = 10) at two times (day 0 and day 14). Skin severity score (A) and body weights (B) were measured on day 0, 14, 24, and 31. Skin severity score was shown as changes of the scores from day 0 of the experimental period. Data represent the means + SEM. *$P < 0.05$, ANOVA followed by the post hoc Bonferroni test.

[0027]

FIG. 7 shows effects of an anti-OSMRβ antibody on histopathological feature of skin lesions in NC/Nga mice. NC/Nga mice were implanted with a hydrogel incorporated with anti-OSMRβ 7D2 monoclonal antibody (B, D, and F) or its isotype control antibody (A, C, and E) at two times (day 0 and day 14). Representative photomicrographs of hematoxylin and eosin-stained sections in the back (A and B), ear (C and D), and face (E and F) were shown. Scale bars = 200 μm.

[0028]

FIG. 8 shows effects of anti-OSMRβ antibody on serum IgE and IL-13 concentration of NC/Nga mice. NC/Nga mice were implanted with a hydrogel incorporated with anti-OSMRβ 7D2 monoclonal antibody (dotted line: n = 4) or its isotype control antibody (solid line: n = 4) at two times (day 0 and day 14). The concentration of serum IgE (FIG. 8(A)) and IL-13 (FIG. 8(B)) were measured on day 0 and 31. *$P < 0.05$, ANOVA followed by the post hoc Bonferroni test.

[0029]

FIG. 9 shows effects of anti-OSMR antibodies on dermatitis of NC/Nga mice. NC/Nga mice were implanted with a hydrogel incorporated with an anti-OSMRβ antibody; 7D2 (dotted lines) or another anti-OSMR antibody; 30-1 (solid line) at two times (day 0 and day 14). Skin severity score was shown as changes of the scores from day 0 of the experimental period. All experimental procedures are same as described in FIG. 6.

[Example]

[0030]

The present inventions can be exemplified in the following Examples but should be not limited thereto.

[0031]

<u>Materials and Methods</u>

1. Antibodies

Antibodies listed below were used in the experiments. PE conjugated anti-Rat Ig k light chain (BD Pharmingen), anti-mouse OSMR (30-1, MBL), goat anti-mIL-31Ra antibody (R&D Systems), purified rat IgG2a k isotype control (R35-95, BD Pharmingen) and rat IgG1 isotype control (Clone 43414, R&D Systems). Isotype control antibody exhibited a low background
5 binding on a variety of mouse tissues. Anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody was purchased from Cell Signaling (Cat#9101).

[0032]

2. Mice

In the present study, 8-week-old C57BL/6J male mice (Clea Japan, Tokyo, Japan) were used.
10 OSMRβ-deficient (OSMR$\beta^{-/-}$) mice were made as described previously (Tanaka et al., 2003). Eight-week-old OSMR$\beta^{+/+}$ and OSMR$\beta^{-/-}$ male mice were used. NC/Nga mice with moderate to severe atopic dermatitis were purchased from SLC (Hamamatsu, Shizuoka, Japan) and kept in conventional conditions until the end-point of the experiments. Twelve-week-old NC/Nga male mice were used in the present study. All mice were kept under a 12-hour light/dark cycle with
15 food and water *ad libitum*. 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 Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23) revised 1978. All efforts were made
20 to minimize the number of animals used and their suffering.

[0033]

3. Generation of Ba/F3 transfectants expressing murine IL-31Ra (mIL-31Ra)

mIL-31Ra gene (SEQ. ID No.3) was cloned from murine dorsal root ganglia and subcloned into a retrovirus vector, pMXs-puro. Retrovirus carrying mIL-31Ra was produced and Ba/F3 cells
25 were infected with the virus. The infected cells were selected with puromycin. Surface expression of mIL-31Ra (SEQ. ID No.4) was confirmed by goat anti-mIL-31Ra antibody staining (R&D Systems) with FACS Calibur (BD).

[0034]

EXAMPLE 1. Generation of anti-murine Oncostatin M receptor β (mOSMRβ) monoclonal
30 antibody

The expression vector carrying soluble form of OSMRβ (SEQ. ID No.5) was cloned into the expression vector which contains the sequences encoding the CD8 signal sequence linked with the FLAG tag and poly-histidine residues. The recombinant soluble OSMRβ (SEQ. ID No.6) was produced in COS7 cells and purified with Ni-NTA and anti-FLAG M2 affinity column.

Wistar rats were immunized with purified soluble mOSMRβ. Lymphocytes were recovered from the lymph nodes and fused with mouse myeloma P3X cells as described in Ogorochi, T et al, 1992. These cells were cultured in HAT medium to select hybridomas (fused cells). Hybridoma supernatants were screened for the production of anti-mOSMRβ antibody with Ba/F3
5 transfectant expressing mOSMRβ (Tanaka et al., 1999). Hybridoma cells producing anti-mOSMRβ antibodies were further cloned by single cell sorting with FACS Aria (Becton). Total 166 hybridoma clones were screened and hybridoma clone called 7D2 was selected as anti-mOSMRβ antibody producer for further analysis (FIG. 2A).

[0035]

10 This hybridoma 7D2 has been deposited as Accession No. FERM ABP-11380 on April 22, 2011 with International Patent Organism Depositary (IPOD), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566 Japan.

[0036]

15 EXAMPLE 2. Purification and preparation of anti-mOSMRβ antibody (7D2 monoclonal antibody) or active fragments thereof

Hybridoma culture supernatant was recovered and isotype of 7D2 monoclonal antibody was evaluated with Rat Monoclonal Antibody Isotyping Test Kit (Serotec). Isotype of 7D2 monoclonal antibody was confirmed as rat IgG1 (FIG. 2B). To purify the antibody, AKTA
20 system (GE healthcare) with protein L column (Pierce) was applied. The culture supernatant from 7D2 hybridoma was passed through the protein L column and the bound antibody was eluted from the column as described in manufacture's protocol. The buffer of 7D2 monoclonal antibody solution was exchanged to Phosphate Buffered Saline (PBS). Purity of the 7D2 monoclonal antibody was evaluated by SDS-PAGE followed with CBB staining (FIG. 2C).
25 The purified 7D2 monoclonal antibody was evaluated with BaF3 transfectant expressing mOSMRβ (BaF3-mOSMRβ). The purified 7D2 monoclonal antibody could stain BaF3-mOSMRβ. Specific binding of 7D2 monoclonal antibody to mOSMRβ was further confirmed by staining of BaF3-mOSMRβ cells and BaF3-mIL31Ra cells. 7D2 monoclonal antibody stained only BaF3-mOSMRβ cells but not BaF3-mIL-31Ra cells (FIG. 2D).

30 [0037]

In further embodiments, the isolated monoclonal antibody is further subjected to conventional methods such like treatment of protease (i.e., papain, pepsin and the like) and linker conjugation in order to obtain active fragment thereof such like a Fab, Fab', $F(ab')_2$, and scFv.

[0038]

EXAMPLE 3. Characterization of anti-mOSMRβ antibody (7D2 monoclonal antibody)
Ba/F3 transfectants expressing mOSMRβ were incubated with purified 7D2 monoclonal antibody or commercially available anti-mOSMR Antibody (clone:30-1, MBL) at several concentrations. After washing the cells with PBS, the cells were incubated with PE conjugated
5 anti-rat IgGs. After washing with PBS, the cells were analyzed with FACS Calibur (Becton). LO cells (Hara et al., 1999) which endogenously express both mOSMRβ and gp130 were stained with the 7D2 or the 30-1 monoclonal antibody as described above and analyzed with FACS Calibur. To evaluate specificity of 7D2 monoclonal antibody to mOSMRβ, 7D2 monoclonal antibody was examined with BaF3 transfectant expressing mIL-31Ra.
10 [0039]
As a consequence, 7D2 monoclonal antibody exhibited better staining to BaF3-mOSMRβ cells compared to commercially available anti-OSMR antibody 30-1, indicating that 7D2 monoclonal antibody had higher affinity to OSMRβ than 30-1. Functional OSM receptor consists of OSMRβ and gp130. LO cells proliferate in response to OSM and express both OSMRβ and
15 gp130 on its cell surface (Hara et al., 1999). LO cells staining suggested that 30-1 had a higher affinity to OSMRβ and gp130 complex than to mOSMRβ monomer (FIGs.3A and 3B). In contrast, 7D2 monoclonal antibody had a less affinity to OSMRβ/gp130 complex than 30-1. Taken together, 7D2 monoclonal antibody is specifically bound to OSMRβ but not to gp130 or IL-31Ra.
20 [0040]
EXAMPLE 4. Ex vivo characterization of anti-mOSMRβ antibody (7D2 monoclonal antibody)
LO transfectants exogenously expressing IL-31Ra were constructed in the same manner as Ba/F3 transfectants. Accordingly, the LO transfectants express the OSM receptor (i.e., combination of mOSMRβ and gp130) as well as the IL-31 receptor (i.e., combination of
25 mOSMRβ and IL-31Ra). Five minutes after incubation with 50ng/ml of OSM or with 50ng/ml of IL-31, the LO transfectants were confirmed to exhibit phosphorylation of Erks, as shown in FIG. 3C.
[0041]
To evaluate the effect of 7D2 monoclonal antibody on the phosphorylation, the phosphorylation
30 of Erks in the LO transfectants was induced by OSM or IL-31 one day after the LO transfectants were per-incubated with 7D2 monoclonal antibody. As a result, approximately 50 % of Erk phosphorylation was reduced by pre-incubation (FIG. 3D).On the other hand, pre-incubation of isotype control antibody did not reduce the Erk phosphorylation. These results indicate that 7D2 monoclonal antibody is useful as an inhibitor of both signal pathways of OSM/OSM receptor

and IL-31/IL31 receptor.

[0042]

EXAMPLE 5. Intradermal injection of cytokines in mice

The mice were housed in individual cages for 3 days. The mice were injected with PBS (40 μl), IL-31 (25-1000 ng/40 μl, Peprotech, Rocky Hill, NJ), or OSM (400 ng/40 μl, R & D Systems,
5 Minneapolis, MN) in the rostral part of the back skin intradermally.

[0043]

Measurement of scratching behavior

The scratching behavior from the hind toes was detected and evaluated using MicroAct (Neuroscience, Tokyo, Japan). The use of MicroAct devices was validated as described
10 elsewhere (Inagaki et al., 2002 & 2003; Takano et al, 2003). Mice were deeply anesthetized with intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg body weight. Under sterile conditions, small Teflon-coated magnets (1 mm diameter, 3 mm length) were implanted subcutaneously into the dorsal side of both hind paws of the mice before recording the scratching behavior. After recovery from the anesthesia, the mice with magnets were placed in
15 the observation chamber (11 cm diameter, 18 cm height) for 1 hour to calm the animals. The extent of the recording time was 2 hours. The MicroAct analysis program was used with the following setting to resister the number of long-lasting (> 1.5 s) scratch events as described previously (Takano et al., 2003): Threshold (V) 0.1, Event Gap (s) 0.2, Max Freq (Hz) 20.0, Min Freq (Hz) 2.0, Min Duration (s) 1.5.

20 [0044]

EXAMPLE 6. Implantation of antibody-incorporated hydrogel in NC/Nga mice

A cross-linked gelatin hydrogel (MedGel; MedGel, Kyoto, Japan) that permitted the controlled release of antibodies was cut and soaked in the solution containing isotype control antibody or anti-OSMRβ antibody (7D2 monoclonal antibody) at 4 °C overnight. The implantation was
25 performed twice with an interval of two weeks. For the first implantation, 20 μg (20 μl) of antibodies were soaked in 2 mg of MedGel, and 100 μg (100 μl) of antibodies were soaked in 10 mg of MedGel for the second implantation. Isotype control antibody- and anti-OSMRβ 7D2 monoclonal antibody-soaked MedGel were then implanted into the back of the nape subcutaneously.

[0045]

30 Clinical assessment

The severity of skin lesion were examined and scored, according to the standard as described by Grimstad et al (2008) with some modifications. The severity of the lesion was graded on a

scale of 0 to 3 (0 = normal skin, 1 = scaly and dry, 2 = nodular lesions, 3 = bloody lesions) for the six parts of the body (right face, left face, right ear, left ear, scalp, and back). The total score (minimum 0, maximum 18) of each mouse was taken as the score for that mouse. Changes of the score from day 0 of the experimental period were shown in the results.

[0046]

5 Measurement of serum IgE and IL-13 levels

To measure serum IgE and IL-13 before and at the end-point of the experiment, mice were bled from the tail vein, and the serum was allowed to separate. Serum IgE and IL-13 was measured using an IgE enzyme-linked immunosorbent assay (ELISA) kit (Morinaga, Tokyo, Japan) and an IL-13 ELISA kit (R & D Systems), respectively, according to the manufacturer's instruction.

[0047]

10 Histological Analysis

At the end-point of the experiment, the mice were deeply anaesthetized with 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). The skins of the back, face, and ear were quickly removed, postfixed in the same fixative at 4 °C for 3 h, and cryoprotected in 20%

15 sucrose in 0.1 M PBS. All specimens were embedded in O.C.T. medium (Sakura Finetek, Torrance, CA), frozen rapidly in cold n-hexane on dry ice, and then were stored at -80 °C. Frozen sections were cut on a cryostat (6-μm thickness) and stained with hematoxylin and eosin.

[0048]

Statistical analysis

The results are shown as the means ± SEM. Statistically significant differences between groups

20 were analyzed by a Student's *t*-test or analysis of variance (ANOVA) followed by the post-hoc Bonferroni test. The criterion for statistical significance was $P < 0.05$.

[0049]

Induction of scratching behavior by IL-31

IL-31 is suggested to be involved in development of atopic dermatitis by inducing itches. IL-31Ra pairs with OSMRβ to form the functional heterodimer for transducing the IL-31

25 signaling (Dreuw et al., 2004). Further, it was demonstrated that IL-31Ra and OSMRβ were co-expressed on neurons at dorsal root ganglia (Bando et al., 2006), suggesting that OSM and/or IL-31 may play roles for the induction of itches at the neurons during atopic dermatitis development. To examine the direct effects of IL-31 on scratching behavior in mice, the present inventors injected IL-31 in the rostral part of the back skin subcutaneously. As shown

30 in FIG. 1A, the number of scratch events by IL-31 began to increase at a dose of 100 ng, reached

a peak at a dose of 400 ng, and was maintained at a dose of 1000 ng. In contrast to IL-31, OSM did not induce any increases in the number of scratch events (FIG. 1A). To investigate the effects of OSMRβ deficiency on IL-31-induced scratching behavior, further the present inventors injected IL-31 to OSMRβ$^{-/-}$ mice. Increased number of scratching behavior by IL-31 in
5 OSMRβ$^{+/+}$ mice was completely abolished in OSMRβ$^{-/-}$ mice (FIG. 1B), indicating that IL-31-induced scratching behavior mediates itches through OSMRβ.

[0050]

Effects of an anti-OSMRβ antibody (7D2 monoclonal antibody) on scratching behavior of NC/Nga mice

Based on the data of FIGs. 1A and 1B, the present inventors hypothesize that blockade of the
10 signaling through OSMR by anti-OSMRβ antibody may ameliorate the pruritus of NC/Nga mice. First, the present inventors examined the effects of an anti-OSMRβ antibody (7D2 monoclonal antibody) on the scratching behavior of NC/Nga mice. The MedGel with anti-OSMRβ 7D2 monoclonal antibody or isotype control antibody was implanted subcutaneously into the back of the nape of NC/Nga mice. As shown in FIG. 4, there were no differences in the number of
15 scratching behavior until 24 days of the experimental period between NC/Nga mice implanted with anti-OSMRβ 7D2 monoclonal antibody (OSMRβ-NC/Nga mice) and NC/Nga mice implanted with isotype control antibody (Isotype-NC/Nga mice). However, the number of scratching behavior was tends to increase in Isotype-NC/Nga mice but not in OSMRβ-NC/Nga mice from 24 days to 31 days of the experimental period (FIG. 4). This suggests that
20 anti-OSMRβ 7D2 monoclonal antibody treatment may play role for the blockage of itches during atopic dermatitis development in some aspects.

[0051]

Effects of anti-OSMRβ antibody (7D2 monoclonal antibody) on dermatitis development of NC/Nga mice

Finally, the present inventors evaluated the effect of anti-mOSMRβ specific antibody (7D2
25 monoclonal antibody) on dermatitis development in NC/Nga mice. The dermatitis score was clinically assessed and severity of skin damage was histologically evaluated. The skin lesions of Isotype-NC/Nga mice were developed throughout the experimental period (FIG. 5, A-C), while the skin lesions of OSMRβ-NC/Nga mice were improved (FIG. 5, D-F). The skin conditions were evaluated by artificial skin severity score, and the results are shown in FIG. 6
30 (A) and (B). The skin severity score in Isotype-NC/Nga mice gradually increased throughout the experimental period. In contrast, the skin severity score in OSMRβ-NC/Nga mice were slightly decreased during 14 days after the first implantation, and drastically decreased after the

second implantation (FIG. 6(A)). In addition, the body weight in OSMRβ-NC/Nga mice was increased compared to that in Isotype-NC/Nga mice (FIG. 6(B)).

[0052]

Histologically, moderate acanthosis and infiltration of inflammatory cells were observed in the back and ear of Isotype-NC/Nga mice. In addition, severe ulcer and infiltration of
5 inflammatory cell were observed in the face of Isotype-NC/Nga mice. On the other hand, these lesions were clearly ameliorated in OSMRβ-NC/Nga mice (FIG. 7). Taken together, these results clearly indicate anti-OSMRβ specific antibody (7D2 monoclonal antibody) can prevent atopic dermatitis development in NC/Nga mice.

[0053]

Effects of anti-OSMRβ antibody (7D2 monoclonal antibody) on serum IgE and IL-13
10 concentration in NC/Nga mice

In NC/Nga mice, skin lesions are usually associated with elevated serum IgE levels and Th2-predominant immunoresponses (Inoue et al., 2007) which usually observed in the patient of atopic dermatitis (Cooper et al., 1994). Before the antibody treatment, there were no significant differences in the levels of serum IgE between OSMRβ-NC/Nga mice and Isotype-NC/Nga mice (FIG. 8 (A) and (B)). After the treatment, contrast to the level of IgE in Isotype-NC/Nga mice, the increase of serum IgE was abolished in OSMRβ-NC/Nga mice (FIG. 8(A)). In addition, the serum IL-13 levels were drastically decreased in OSMRβ-NC/Nga mice after the treatment while this cytokine levels were increased in Isotype-NC/Nga mice (FIG. 8(B)). These results indicate that inflammatory response during atopic dermatitis development was also prevented by the treatment with anti-OSMRβ 7D2 monoclonal antibody.

[0054]

Comparison of an anti-OSMRβ antibody 7D2 and anti-OSMR complex antibody 30-1 on dermatitis development of NC/Nga mice

The present inventors evaluated the effect of 7D2 and 30-1 on dermatitis development in NC/Nga mice. The severity of skin damage was histologically evaluated. The skin severity score
15 in 30-1 antibody implanted mice gradually increased throughout the experimental period. In contrast, the skin severity score in 7D2 implanted mice were drastically decreased (FIG. 9). As described above, 7D2 monoclonal antibody is specifically bound to OSMRβ but not to gp130 or IL-31Ra. In contrast, 30-1 monoclonal antibody has higher affinity to OSMRβ/gp130 complex than that to OSMRβ monomer. Actually, 30-1 antibody barely bound to OSMRβ monomer (FIG.
20 3). These data suggest that the antibodies to OSMRβ/gp130 complex, which are like 30-1, don't block atopic dermatitis development and only OSMRβ specific antibody as 7D2 can prevent skin

inflammation under the dermatitis development.

[0055]

Taken together, the present inventors' findings indicate that anti-OSMRβ specific antibody is a good candidate for the treatment of atopic dermatitis. It has been demonstrated that anti-IL-31 antibody only reduced scratching behavior in same mice model and dermatitis development was
5 not improved by anti-IL-31 antibody treatment. This suggests that blockage of IL-31 alone is not enough to prevent dermatitis development. Both IL-31 and OSM shares OSMRβ as receptor signaling component. Thus, the role of both IL-31 and OSM will be regulated by targeting to OSMRβ with anti-OSMRβ antibody.

[0056]

In another embodiment, the present inventions provide a method for isolation and identification of an alternative molecule possessing more potent binding or inhibition activities in comparison with 7D2 monoclonal antibody, comprising the following steps:

(1) preparing and incubating a candidate molecule with the cells expressing OSMRβ alone, OSM receptor, IL-31 receptor, and/or combination thereof, in the presence of 7D2 monoclonal antibody,

wherein said molecule may include small chemical compounds; proteins including antibodies as well as small peptide consisting of approximately 5 ~ 20 a.a.; and nucleic acids including aptamers, but should not be restricted thereto;

wherein the cells expressing OSMβ alone may be a BaF3 transfectant expressing OSMRβ; the cells expressing OSM receptor may be LO cells; and the cells expressing OSM receptor and IL-31 receptor may be a LO transfectant expressing IL-31Ra; and

(2) indirectly investigating whether said candidate molecule can competitively bind to the cells by measuring the binding of 7D2 monoclonal antibody to the cells

[0057]

In another embodiment, the present inventions provide a method for isolation and identification of an alternative molecule possessing more potent inhibition activity in comparison with 7D2 monoclonal antibody, comprising the following steps:

(1) preparing and incubating a candidate molecule with the cells expressing OSM receptor, IL-31 receptor or both

wherein said molecule may include small chemical compounds; proteins including antibodies as well as small peptide consisting of approximately 5 ~ 20 a.a.; and nucleic acids including aptamers, but should not be restricted thereto;

wherein the cells expressing OSM receptor may be LO cells; and the cells

expressing OSM receptor and IL-31 receptor may be a LO transfectant expressing IL-31Ra; and (2) investigating whether said candidate molecule can more significantly suppress the phosphorylation activity induced by signal transduction from OSM to OSM receptor and/or from IL-31 to IL-31 receptor in comparison with 7D2 monoclonal antibody.

[Industrial Applicability]

[0058]

Consequently, OSMRβ specific 7D2 monoclonal antibody clearly prevented dermatitis development in mice model, suggesting that anti-OSMRβ antibody is a new potential therapeutic approach for the treatment of atopic dermatitis during the onset of clinical skin manifestations.

[0059]

Cross Reference

The whole contents of the following cited references are included in the disclosure of this Description.

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5 aorta-gonad-mesonephros region. *Immunity11: 567-78.*

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10 Dieu-Nosjean MC, Meller S, Rieker J, Steinhoff M, Hoffmann TK, Ruzicka T, Zlotnik A, Homey B (2006) IL-31: A new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol 117: 411-417.*

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(13) Tanaka, M., Hara, T., Copeland, N. G., Gilbert, D. J., Jenkins, N. A. and Miyajima, A., Reconstitution of the functional mouse oncostatin M (OSM) receptor: molecular cloning of the mouse OSM receptor beta subunit. *Blood* 1999. 93: 804-815.

[Kind of Document] Claims

1. A hybridoma cell line deposited as Accession No. FERM ABP-11380.

2 A monoclonal antibody against oncostatin M specific receptor beta subunit, obtained from the hybridoma according to claim 1.

3. An active fragment of the antibody according to claim 2, selected from the group consisting of a Fab, Fab', $F(ab')_2$, and scFv.

4 An inhibitor against the signal pathways between OSM and OSM receptor and between IL-31 and IL-31 receptor, comprising the monoclonal antibody according to claim 2 or the fragment according to claim 3.

5. A medicament for treating atopic dermatitis comprising the monoclonal antibody according to claim 2 or the fragment according to claim 3.

6. Use of the monoclonal antibody according to claim 2 or the fragment according to claim 3 for manufacturing the medicament for treating atopic dermatitis.

7 A method for treating atopic dermatitis comprising a therapeutically effective amount of the monoclonal antibody according to claim 2 or the fragment according to claim 3 with a pharmaceutically acceptable carrier, excipient or diluent.

8 A method for isolation and identification of a molecule possessing more potent binding activity against OSMRβ as well as against OSM receptor consisting of OSMRβ and gp130 in comparison with the monoclonal antibody according to claim 2, comprising the following steps:

(1) preparing and incubating said molecule with (a) the cells expressing OSMRβ and expressing no gp130, and with (b) the cells expressing OSM receptor, in the presence of the monoclonal antibody according to claim 2; and

(2) indirectly investigating whether said molecule can competitively bind to both of said cells by measuring the binding of the monoclonal antibody according to claim 2 to both of said cells.

9 A method for isolation and identification of a molecule possessing more potent inhibition activities against OSM and/or IL-31 signal pathways in comparison with the monoclonal antibody according to claim 2, comprising the following steps:

(1) preparing and incubating a candidate molecule with the cells expressing OSM receptor and IL-31 receptor; and

(2) investigating whether said candidate molecule can more significantly suppress the phosphorylation activity induced by signal transduction from OSM to OSM receptor and/or from IL-31 to IL-31 receptor in comparison with the monoclonal antibody according to claim 2.

FIG.1A

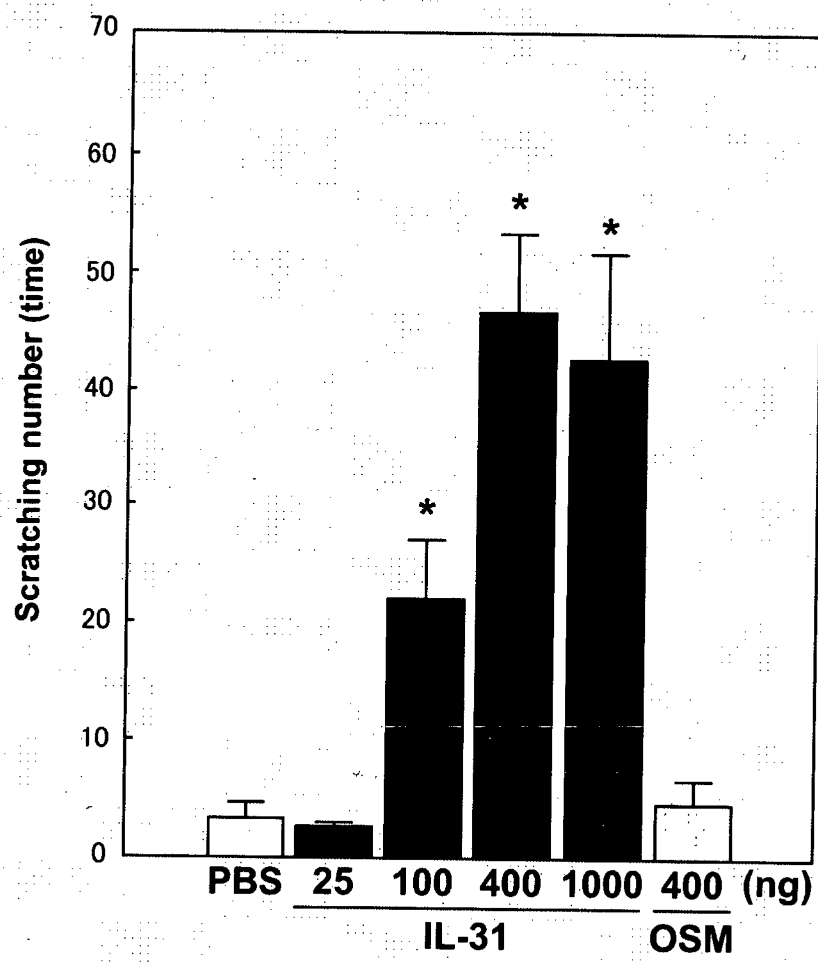
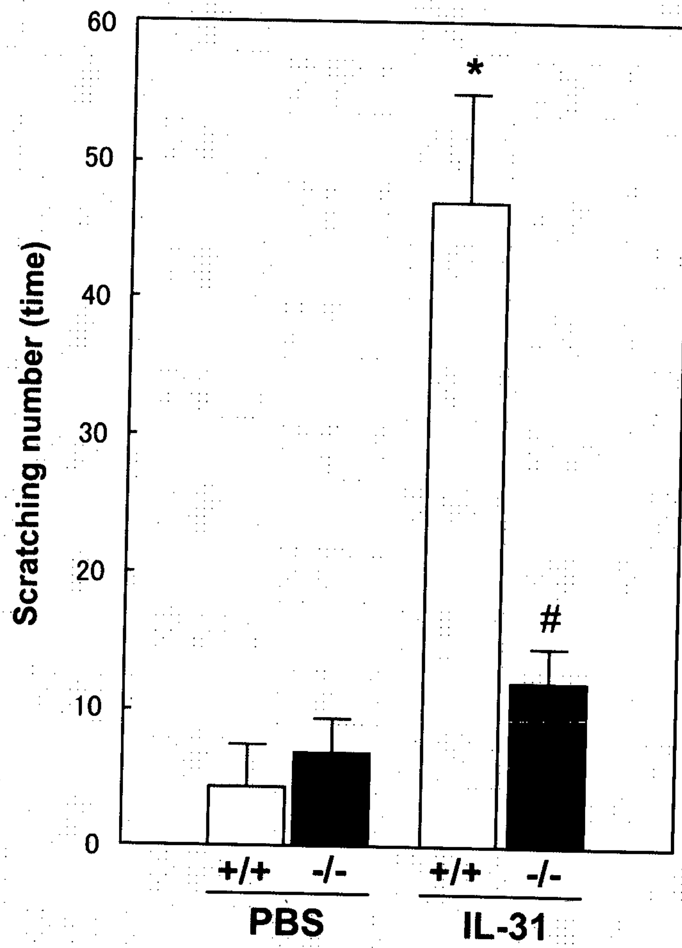


FIG.1B



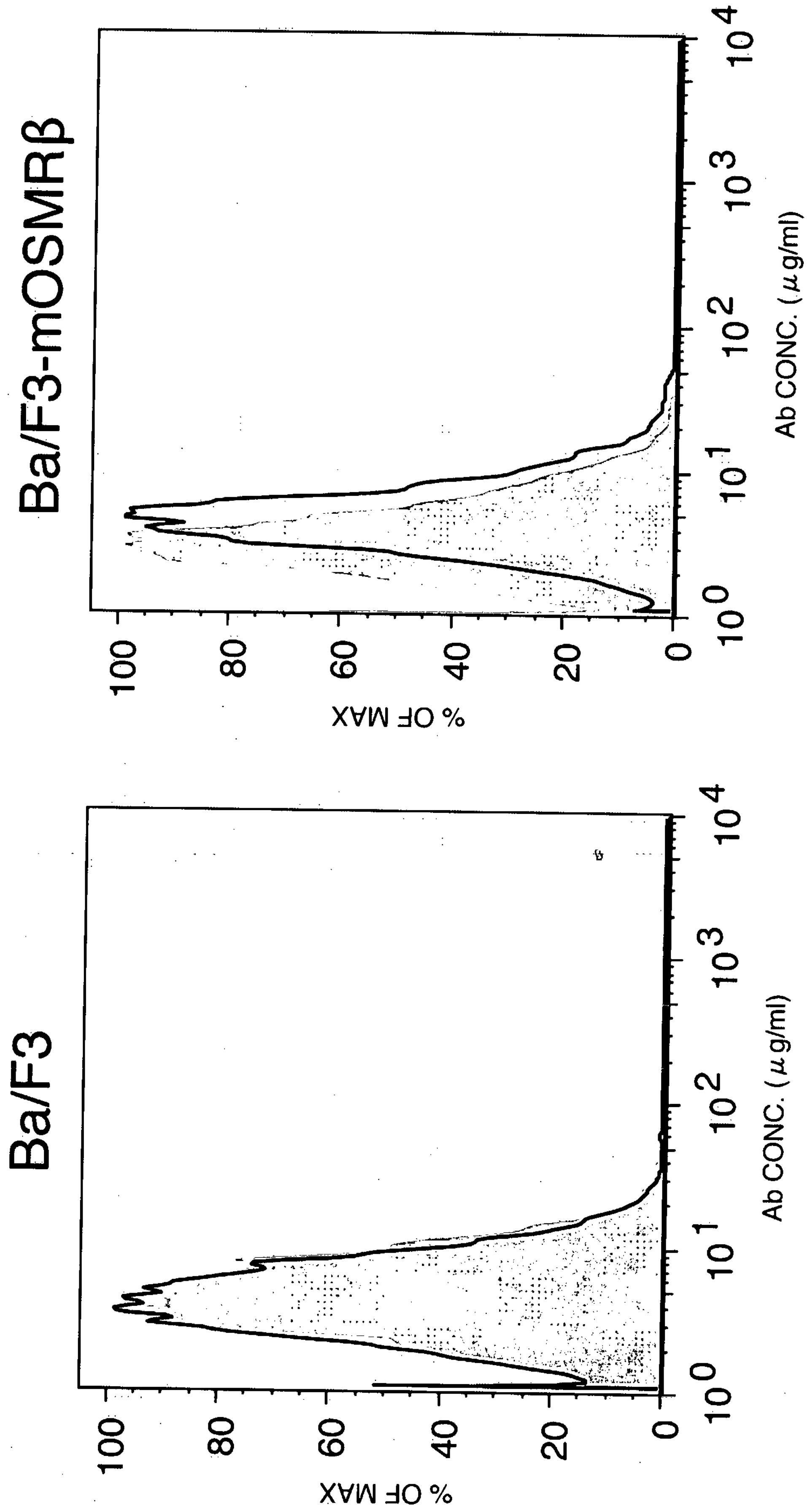


FIG.2A

FIG.2B

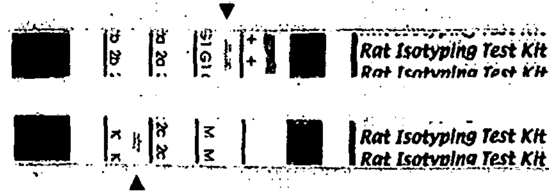
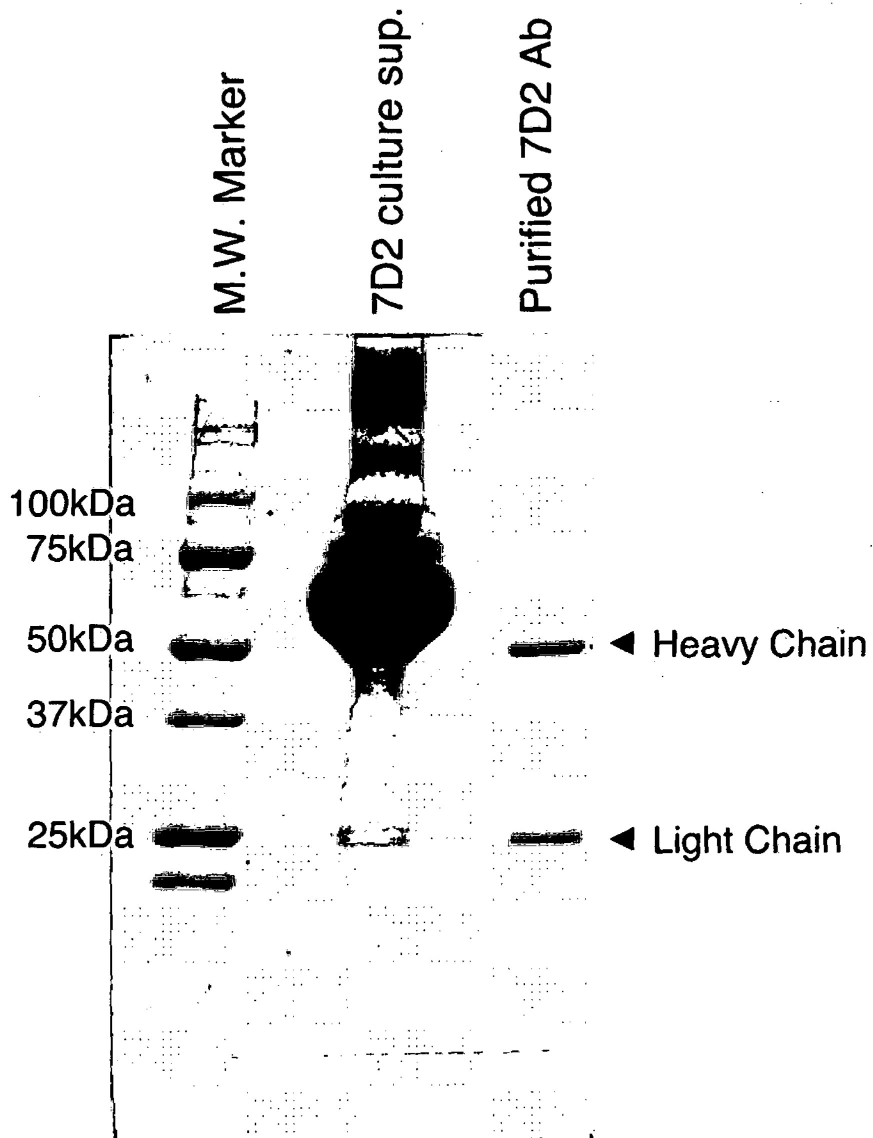
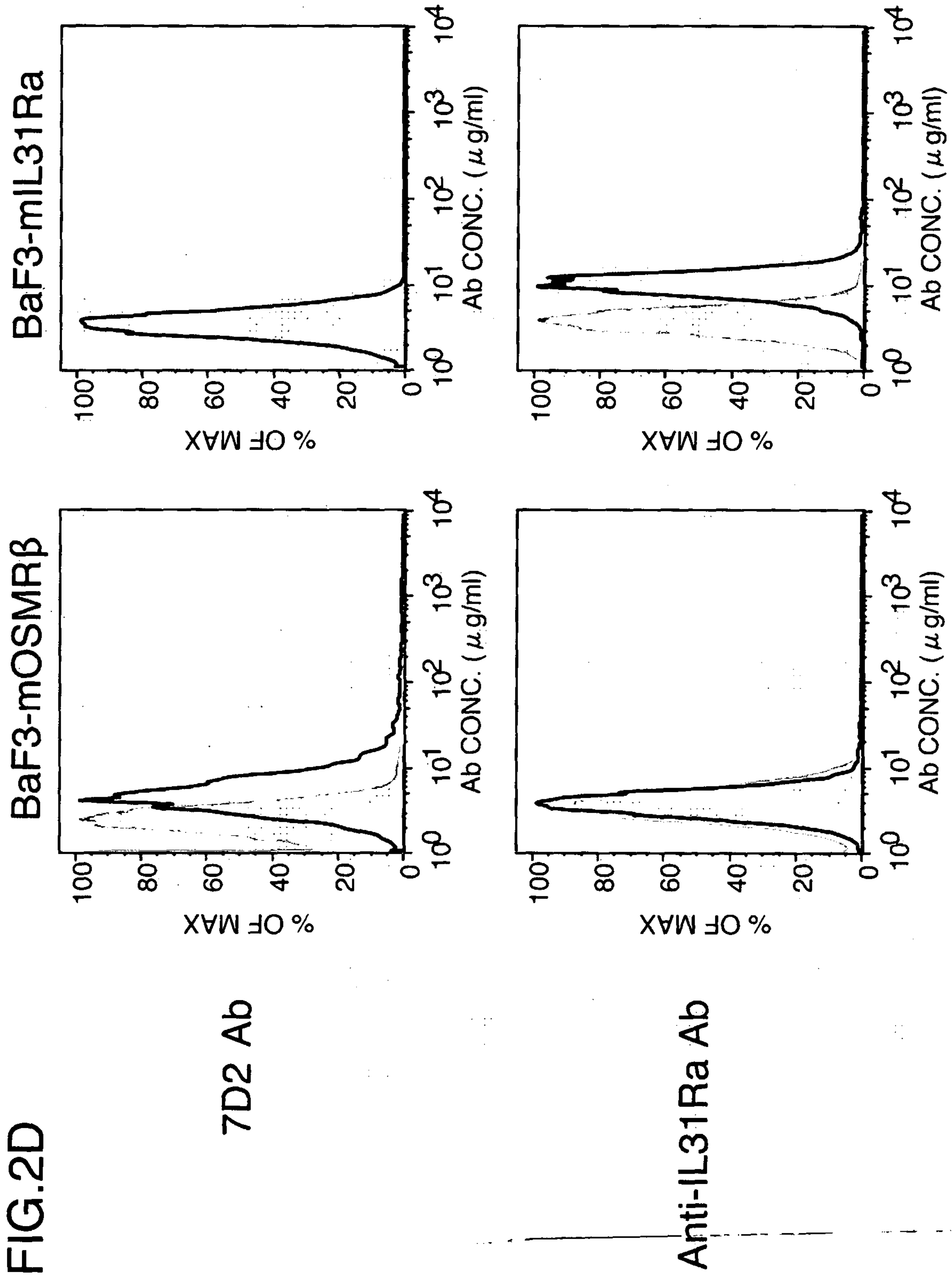


FIG.2C





BaF3 transfectans were stained with purified 7D2 or anti-IL31Ra Ab

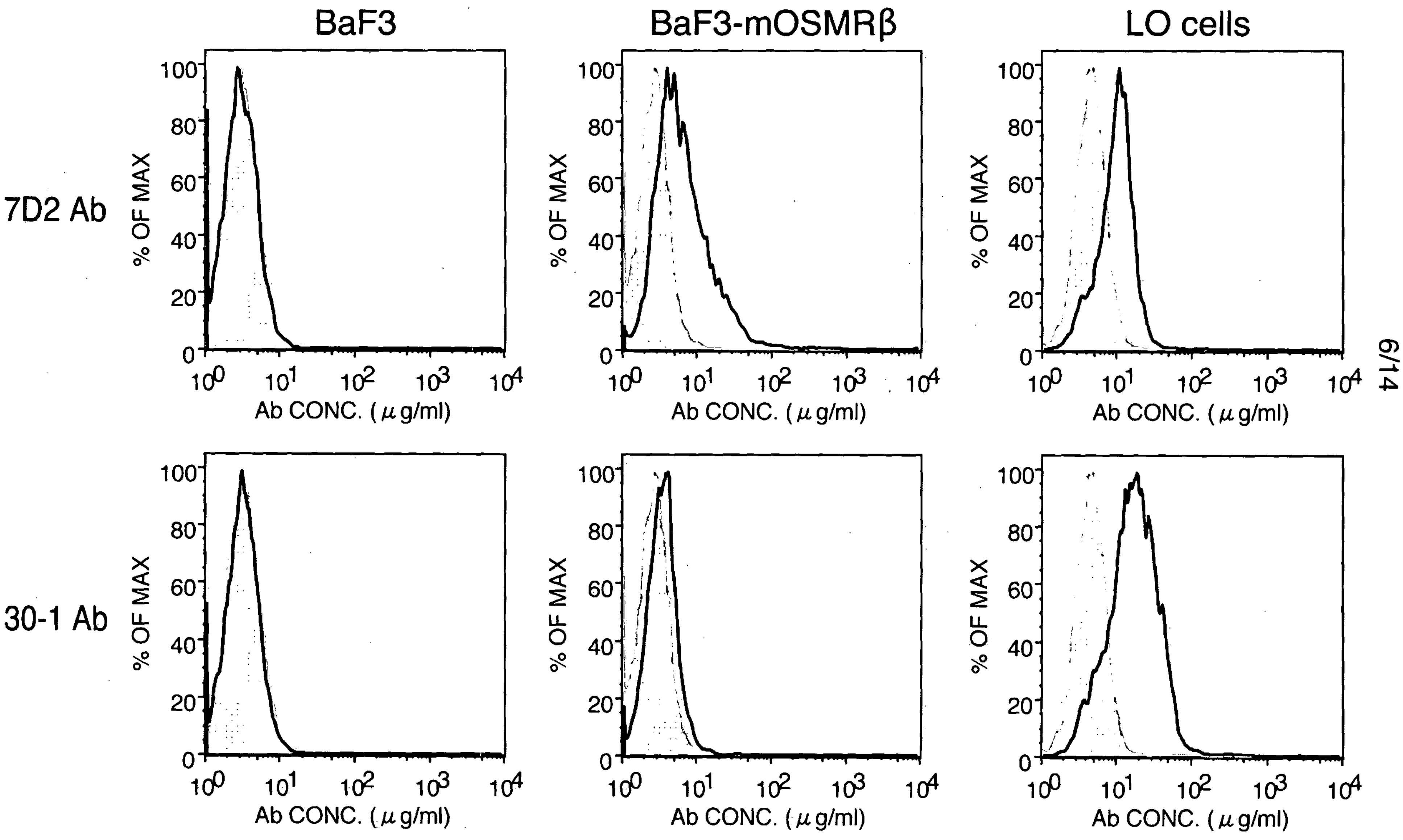


FIG.3A
BaF3
BaF3-mOSMRβ
LO cells
7D2 Ab
30-1 Ab
% OF MAX
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
Ab CONC. (μg/ml)

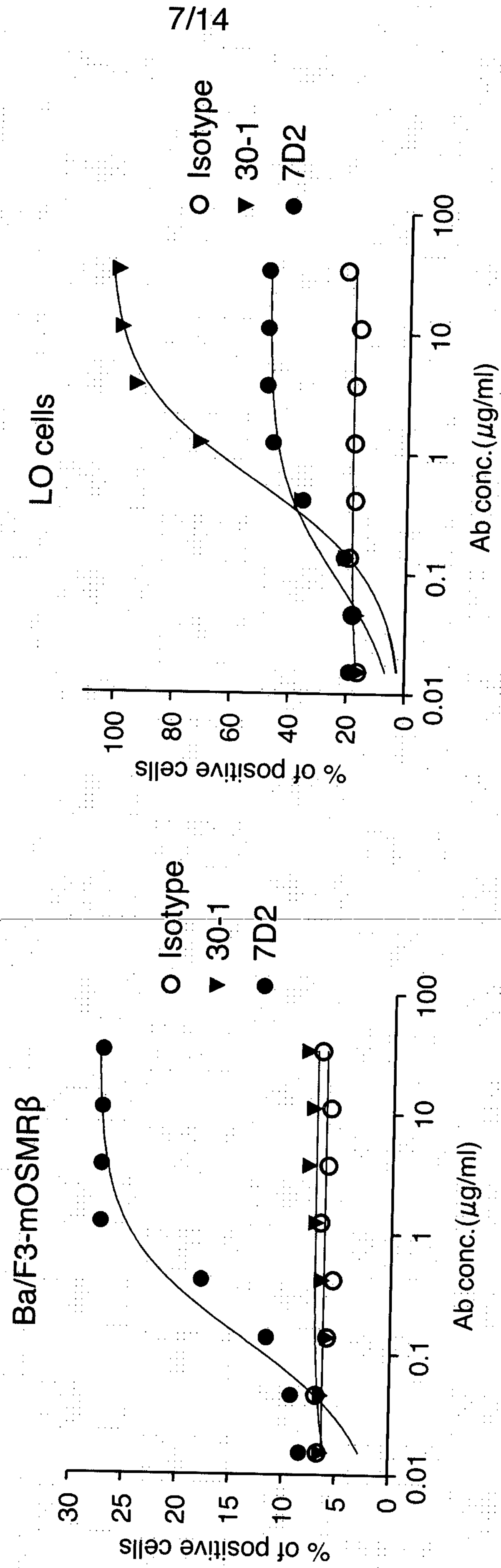


FIG.3B
LO cells
Isotype
30-1
7D2
% of positive cells
Ab conc.(μg/ml)
0
20
40
60
80
100
0.01
0.1
1
10
100
Ba/F3-mOSMRβ
Isotype
30-1
7D2
% of positive cells
Ab conc.(μg/ml)
0
5
10
15
20
25
30
0.01
0.1
1
10
100

FIG.3C

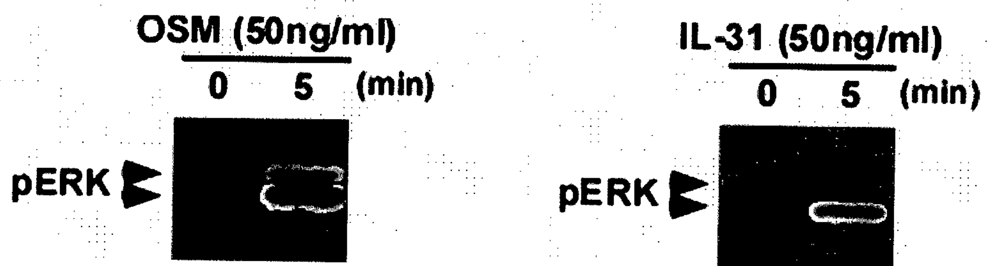


FIG.3D

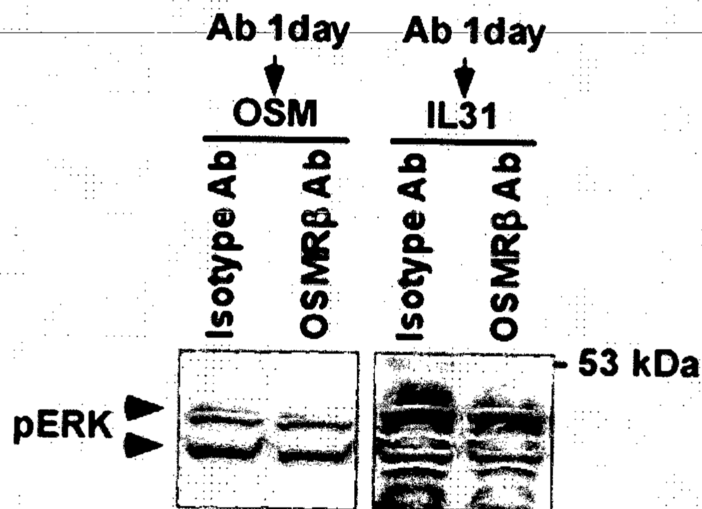


FIG.4

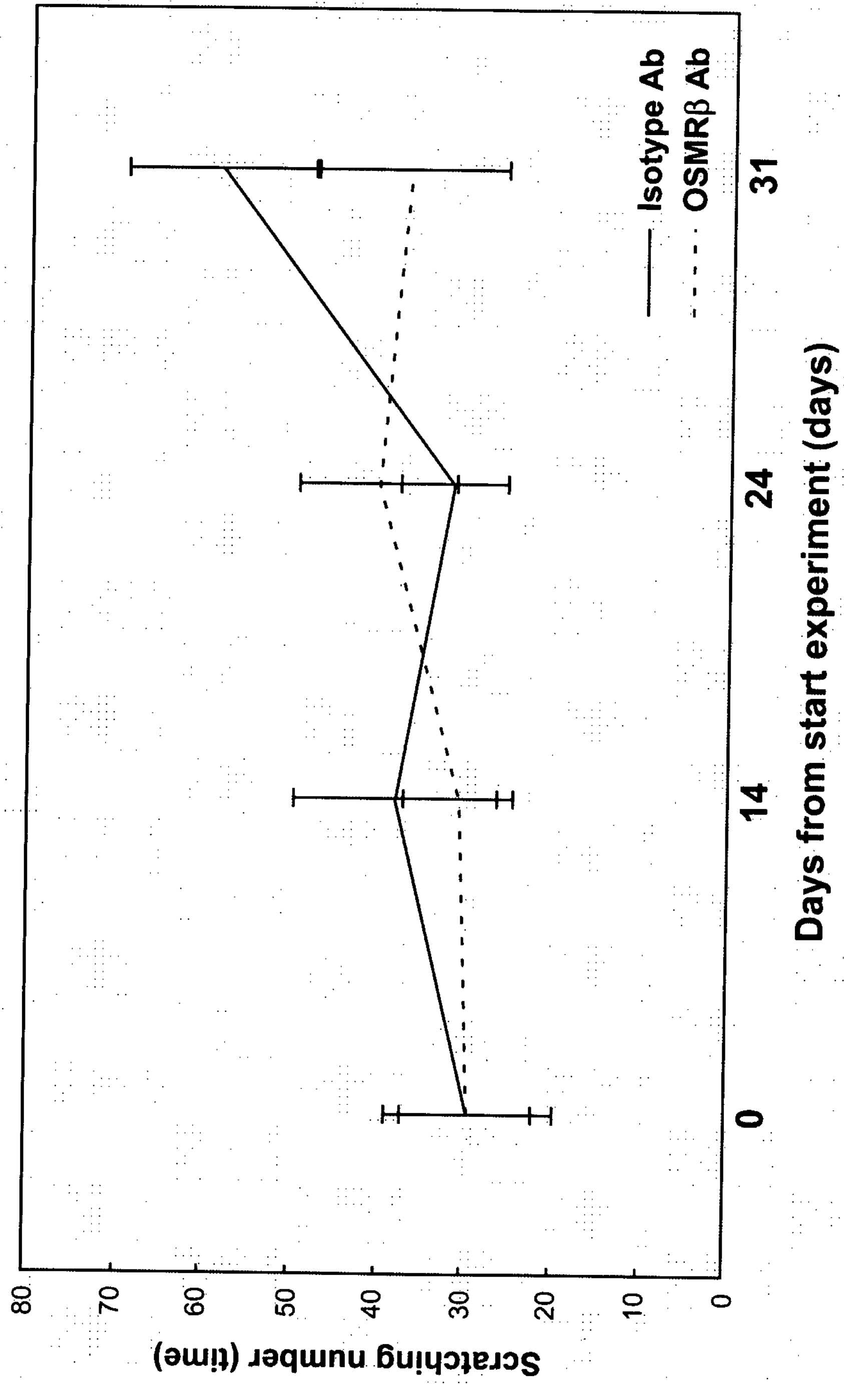


FIG.5

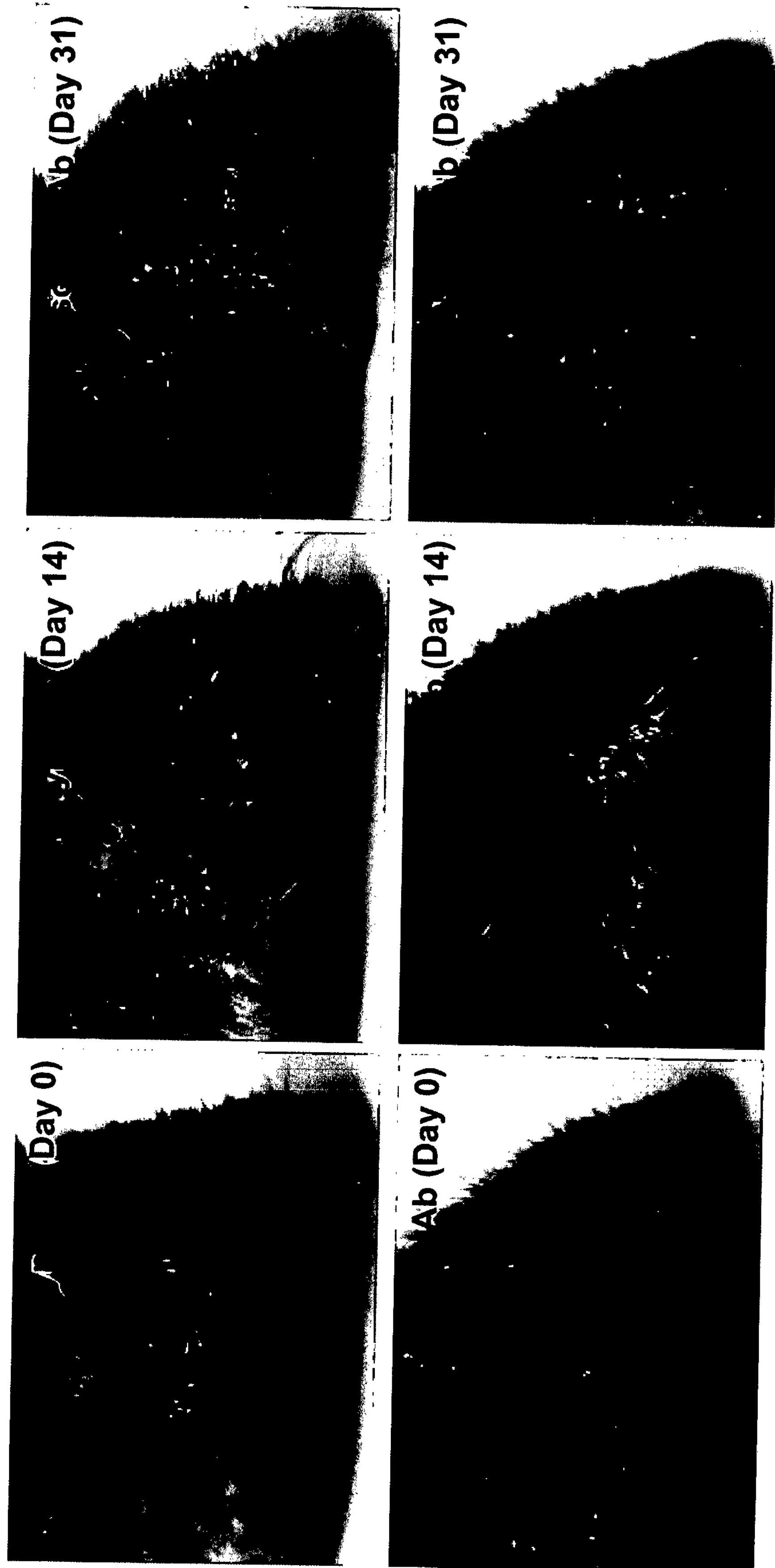
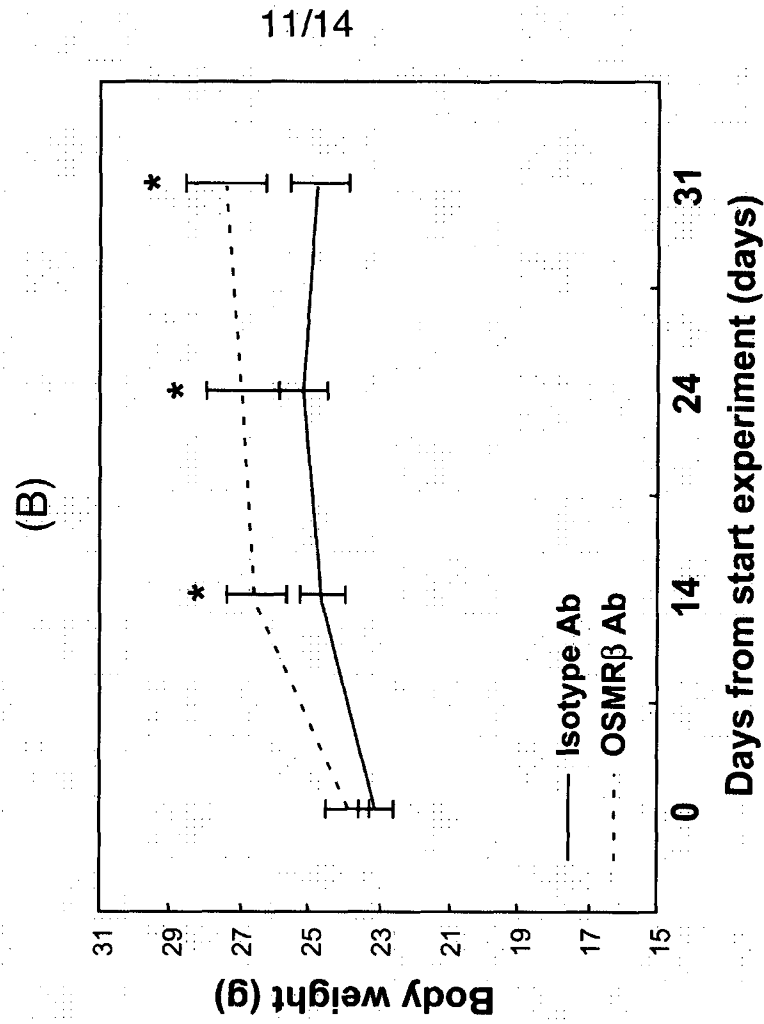


FIG.6



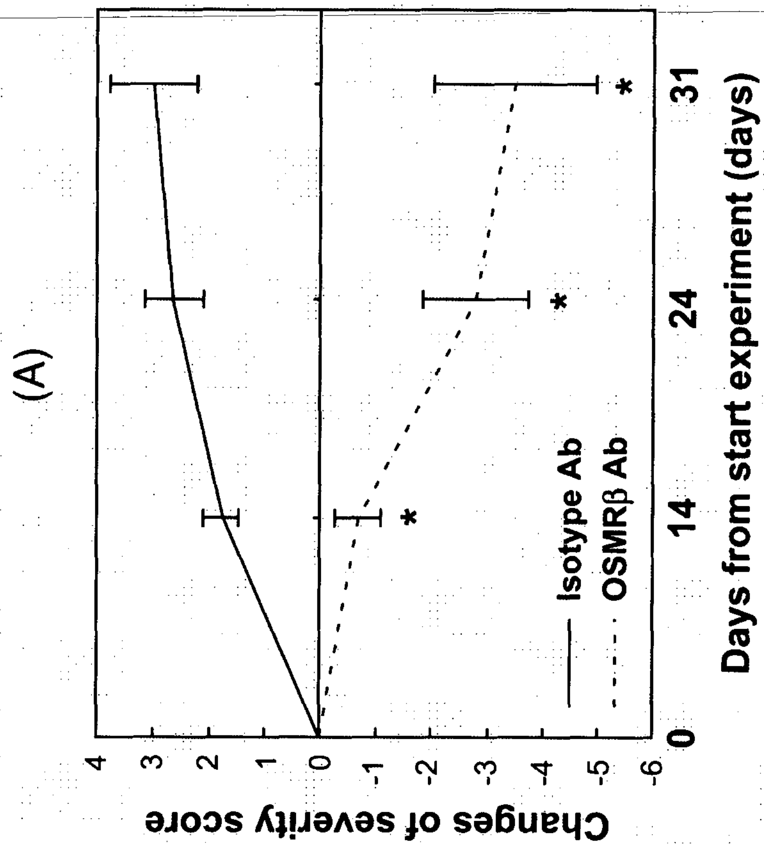
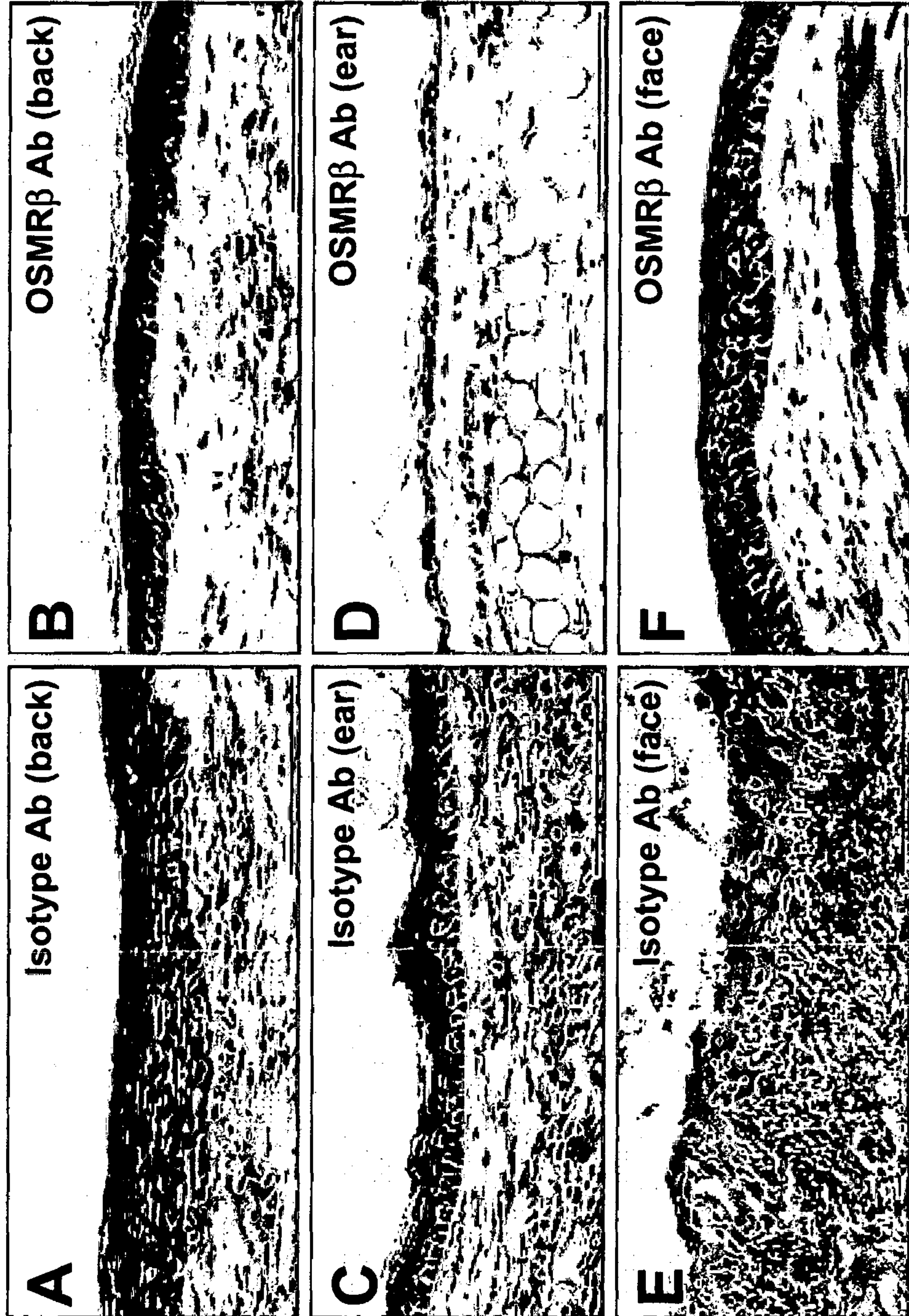


FIG.7



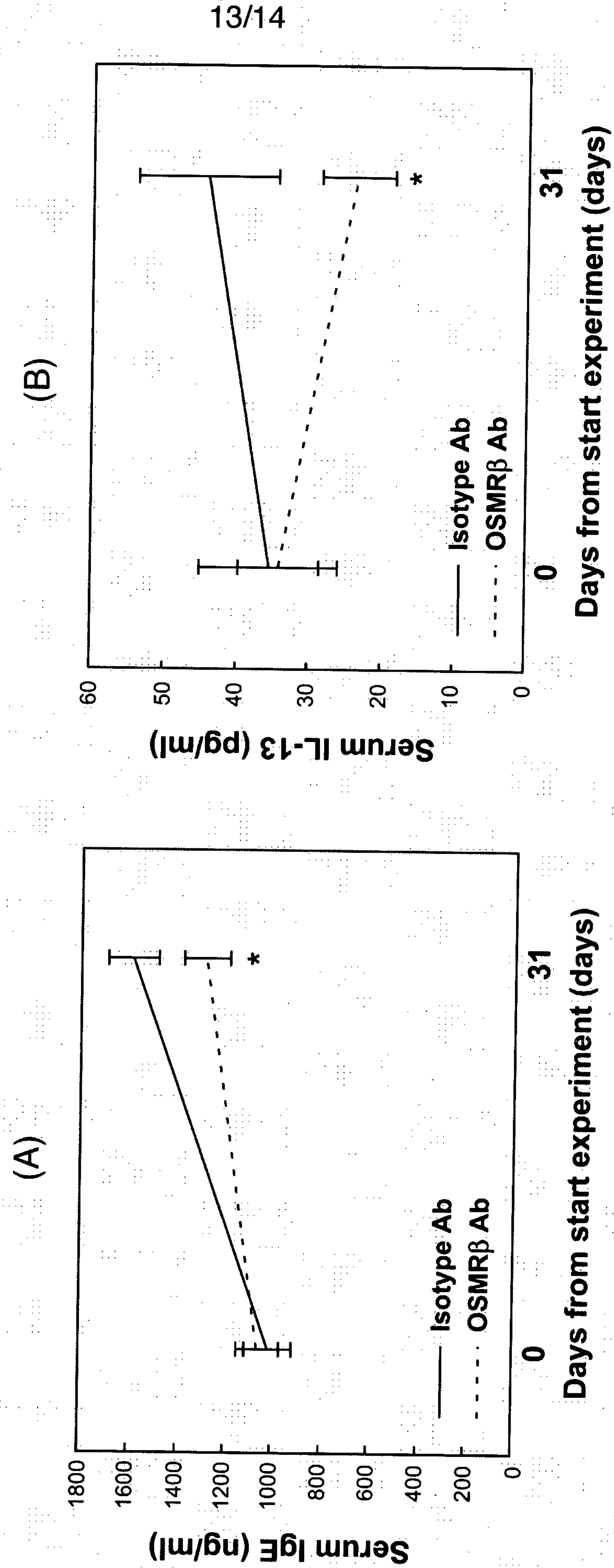


FIG.8
(A)
Serum IgE (ng/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
Isotype Ab
OSMRβ Ab
*
0
31
Days from start experiment (days)
(B)
Serum IL-13 (pg/ml)
60
50
40
30
20
10
0
Isotype Ab
OSMRβ Ab
*
0
31
Days from start experiment (days)

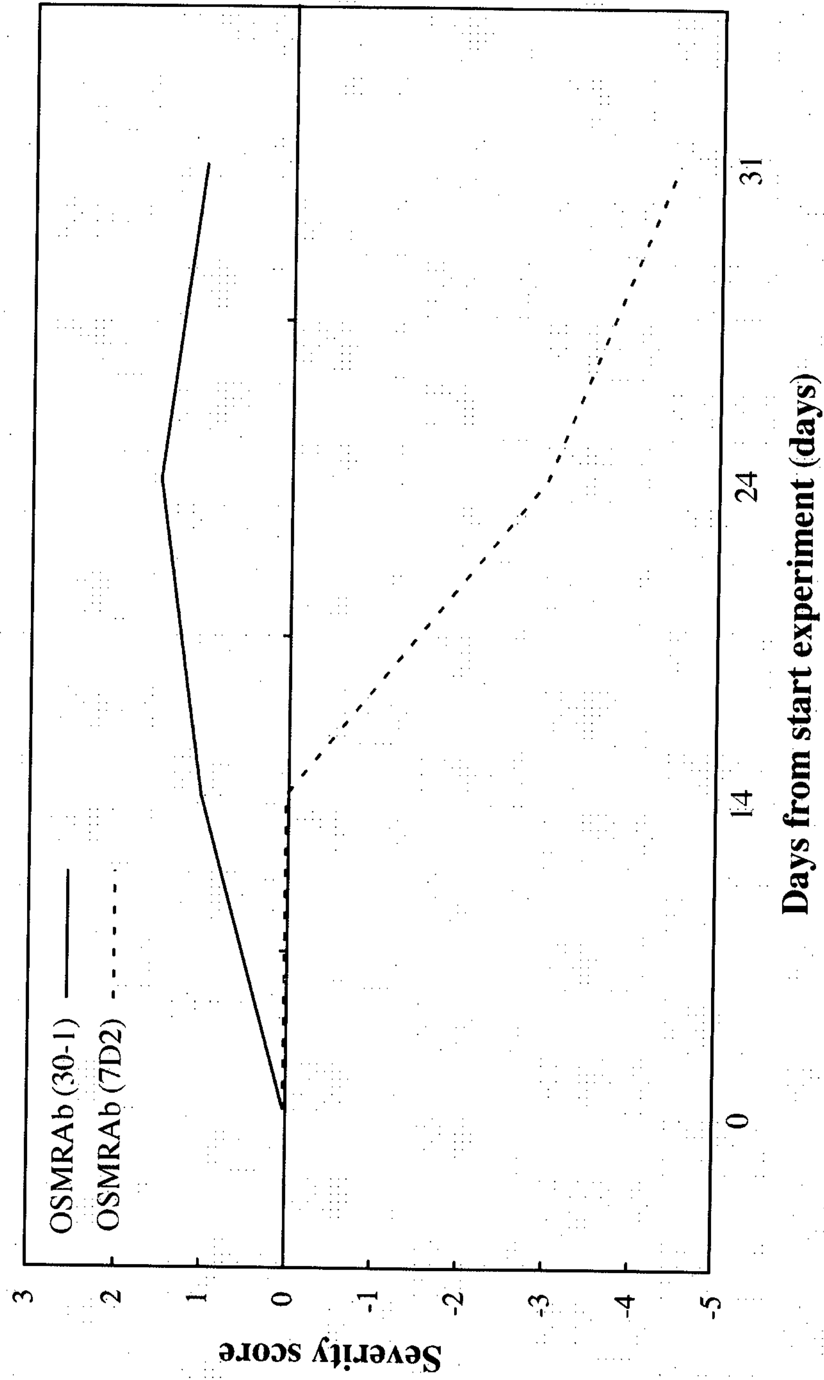


FIG.9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/063734

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C07K16/28(2006.01)i, A61K39/395(2006.01)i, A61P17/00(2006.01)i, C12N15/02(2006.01)i, C12P21/08(2006.01)n

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C07K16/00-16/46, A61K39/395, A61P17/00, C12N15/00-15/90, C12P21/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2013
Registered utility model specifications of Japan 1996-2013
Published registered utility model applications of Japan 1994-2013

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAplus/MEDLINE/EMBASE/BIOSIS/WPIDS(STN), JSTPlus/JMEDPlus/JST7580(JDreamIII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 1995/033059 A2 (IMMUNEX CORPORATION) 1995.12.07 & JP 10-501131 A & US 5783672 A & US 5891997 A & US 5925740 A & US 6010886 A & US 6524817 B1 & US 2003/0109003 A1 & US 2005/0260197 A1 & EP 760857 A1	1-6,8-9
Y	MOSLEY,B.et al., Dual Oncostatin M (OSM) Receptors. J.Biol.Chem.,1996,Vol.271,No.50,p.32635-32643	1-6,8-9

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28.05.2013	11.06.2013

Name and mailing address of the ISA/JP	Authorized officer
Japan Patent Office	Kenji MIHARA 4B 2937
3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Telephone No. +81-3-3581-1101 Ext. 3448

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/063734

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BANDO,T.et al., COMPLETE OVERLAP OF INTERLEUKIN-31 RECEPTOR A AND ONCOSTATIN M RECEPTOR ß IN THE ADULT DORSAL ROOT GANGLIA WITH DISTINCT DEVELOPMENTAL EXPRESSION PATTERNS. Neuroscience,2006,Vol.142,p.1263-1271	1-6,8-9
A	WO 2006/084092 A2 (RAVEN BIOTECHNOLOGIES, INC.) 2006.08.10 & JP 2008-532488 A & US 2006/0171951 A1 & US 2009/0269340 A1 & EP 1846455 A2	1-6,8-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/063734

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   - [ ] on paper
   - [x] in electronic form

   b. (time)

   - [ ] in the international application as filed
   - [x] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purposes of search

2. [x] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/063734

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 7
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claim 7 relates to a method for treatment of the human or animal body by therapy, which does not require an international search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and Rule 39.1(iv).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.