comprising an amino acid sequence comprising amino acid residues 20 to 140 of an amino acid sequence represented by SEQ ID NO: 53 and a light chain sequence containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 133 of an amino acid sequence represented by SEQ ID NO: 63.

(13) The antibody or a functional fragment of the antibody according to (10), characterized by containing a heavy chain sequence containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of an amino acid sequence represented by SEQ ID NO: 55 and a light chain sequence containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 133 of an amino acid sequence represented by SEQ ID NO: 65.

(14) The antibody or a functional fragment of the antibody according to (10), characterized by containing a heavy chain sequence containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of an amino acid sequence represented by SEQ ID NO: 55 and a light chain sequence containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to ~~133~~132 of an amino acid sequence represented by SEQ ID NO: 67.

(15) The antibody or a functional fragment of the

(50) The polynucleotide according to (46), characterized by containing a polynucleotide comprising a nucleotide sequence comprising nucleotides 58 to 420 of a nucleotide sequence represented by SEQ ID NO: 54, and a polynucleotide comprising a nucleotide sequence comprising nucleotides 61 to ~~399~~396 of a nucleotide sequence represented by SEQ ID NO: 66.

(51) The polynucleotide according to (46), characterized by containing a polynucleotide comprising a nucleotide sequence comprising nucleotides 58 to 420 of a nucleotide sequence represented by SEQ ID NO: 56, and a polynucleotide comprising a nucleotide sequence comprising nucleotides 61 to 399 of a nucleotide sequence represented by SEQ ID NO: 68.

(52) The polynucleotide according to (46), characterized by containing a polynucleotide comprising a nucleotide sequence comprising nucleotides 58 to 420 of a nucleotide sequence represented by SEQ ID NO: 58, and a polynucleotide comprising a nucleotide sequence comprising nucleotides 61 to 399 of a nucleotide sequence represented by SEQ ID NO: 70.

(53) The polynucleotide according to (46), characterized by containing a polynucleotide comprising a nucleotide sequence comprising nucleotides 58 to 420 of a nucleotide sequence represented by SEQ ID NO: 98, and a polynucleotide comprising a nucleotide sequence comprising nucleotides 61 to 399 of a nucleotide sequence represented by SEQ ID NO: 68.

(54) The polynucleotide according to (46), characterized

Incidentally, the rat #32A1 antibody and #32A1 human chimeric antibody in the drawing are positive controls common to Figs. 46 and 47.

[Fig. 47] Fig. 47 shows graphs depicting, by the enzymatic activity of TRAP, the inhibition of mouse osteoclast formation by the addition of a humanized rat anti-mouse Siglec-15 antibody (h#32A1-H1-1/L2-16 or h#32A1-H-1/L2-15).

[Fig. 48] Fig. 48 shows graphs depicting the inhibition of the bone resorption activity of normal human osteoclasts by the addition of a humanized rat anti-mouse Siglec-15 antibody (h#32A1-T1, h#32A1-T2, h#32A1-T3, h#32A1-T4, h#32A1-T5, or h#32A1-T6). Incidentally, the rat #32A1 antibody and #32A1 human chimeric antibody in the drawing are positive controls common to Figs. 48 and 49.

[Fig. 49] Fig. 49 shows graphs depicting the inhibition of the bone resorption activity of normal human osteoclasts by the addition of a humanized rat anti-mouse Siglec-15 antibody (h#32A1-H1-1/L5, h#32A1-H5/L5, h#32A1-H1-1/L2-16, or h#32A1-H1-1/L~~1~~2-1~~5~~).

[Fig. 50] Fig. 50 is a thermogram obtained for determining the thermal stability of the h#32A1-H5/L5 antibody.

[Fig. 51] Fig. 51 is a thermogram obtained for determining the thermal stability of the h#32A1-H1-1/L5 antibody.

[Fig. 52] Fig. 52 is a thermogram obtained for determining the thermal stability of the h#32A1-H1-1/L12-15 antibody.

[Fig. 53] Fig. 53 is a thermogram obtained for determining the thermal stability of the h#32A1-H1-1/L2-16 antibody.

[Fig. 54] Fig. 54 shows a nucleotide sequence of h#32A1-H1-1 and the amino acid sequence thereof.

[Fig. 55] Fig. 55 shows a nucleotide sequence of h#32A1-H5 and the amino acid sequence thereof.

[Fig. 56] Fig. 56 shows a nucleotide sequence of h#32A1-L2-15 and the amino acid sequence thereof.

[Fig. 57] Fig. 57 shows a nucleotide sequence of h#32A1-L2-16 and the amino acid sequence thereof.

Mode for Carrying Out the Invention

[0012]

The term “gene” as used herein includes not only DNA, but also mRNA, cDNA, and cRNA.

[0013]

The term “polynucleotide” as used herein is used with the same meaning as a “nucleic acid” and also includes DNA, RNA, probes, oligonucleotides, and primers.

[0014]

The terms “polypeptide” and “protein” as used herein are

heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of SEQ ID NO: 55 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to ~~133~~132 of SEQ ID NO: 67, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of SEQ ID NO: 57 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 133 of SEQ ID NO: 69, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of SEQ ID NO: 59 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 133 of SEQ ID NO: 71, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of SEQ ID NO: 99 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 133 of SEQ ID NO: 69, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of SEQ ID NO: 101 and a light chain containing a light chain variable region comprising an amino acid sequence

Siglec-15 monoclonal antibody #32A1 on osteoclast differentiation of mouse bone marrow nonadherent cells (stimulation with TNFα)

By using the rat anti-mouse Siglec-15 monoclonal antibody #32A1 prepared in Example 6 and the chimeric antibody of the rat anti-mouse Siglec-15 monoclonal antibody #32A1 prepared in Example 19, an effect on osteoclast differentiation of mouse bone marrow nonadherent cells by stimulation with TNFα was examined. Mouse bone marrow nonadherent cells prepared by the method of Example 8 were prepared at 1.5×10^5 cells/ml in α-MEM medium containing 10% fetal bovine serum (FBS), 10 ng/ml of M-CSF, and 2 ng/ml of TGF-β (manufactured by R&D Systems, Inc.), the resulting cell preparation was seeded in each well of a 96-well plate in an amount of 200 μl and the cells were cultured for 2 days in a CO_2 incubator. The old culture solution in the 96-well plate was removed, and 100 μl of MEM-α medium was added to each well, the 100 μl of MEM-α medium containing 10% FBS to which recombinant human TNFα (manufactured by R&D Systems, Inc.) and M-CSF had been added to give final concentrations of 30 ng/ml and 10 ng/ml, respectively. To the cell culture solution, the rat anti-mouse Siglec-15 monoclonal antibody #32A1 prepared in Example ~~9~~6 or the chimeric antibody of the rat anti-mouse Siglec-15 monoclonal antibody #32A1 prepared in Example 19 was added at a concentration of from 0.125 to 4 μg/ml, and the cells

was taken as a thermal denaturation midpoint temperature (Tm) in the Fab region, the Tm value of h#32A1-H5/L5 was 81.2°C, the Tm value of h#32A1-H1-1/L5 was 84.1°C, the Tm value of h#32A1-H1-1/L2-15 was 80.0°C,, and the Tm value of h#32A1-H1-1/L2-16 (IgG2) was 77.9°C.

Industrial Applicability

[0339]

The chimeric or humanized anti-Siglec-15 antibody of the invention has an ability to inhibit osteoclast differentiation or bone resorption activity, and a pharmaceutical composition containing the anti-Siglec-15 antibody can be a therapeutic or preventive agent for a disease of abnormal bone metabolism.

Accession Number

[0340]

FERM BP-10999