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Development of Sensitive Anti-Mouse CCR5 Monoclonal Antibodies Using the N-Terminal Peptide Immunization

Rena Ubukata,* Hiroyuki Suzuki,* Tomohiro Tanaka, Guanjie Li, Mika K. Kaneko, and Yukinari Kato

One of the G protein-coupled receptors, C-C chemokine receptor 5 (CCR5), is an important regulator for the activation of T and B lymphocytes, dendritic cells, natural killer cells, and macrophages. Upon binding to its ligands, CCR5 activates downstream signaling, which is an important regulator in the innate and adaptive immune response through the promotion of lymphocyte migration and the secretion of proinflammatory cytokines. Anti-CCR5 monoclonal antibodies (mAbs) have been developed and evaluated in clinical trials for tumors and inflammatory diseases. In this study, we developed novel mAbs for mouse CCR5 (mCCR5) using the N-terminal peptide immunization. Among the established anti-mCCR5 mAbs, C_5Mab-4 (rat IgG_{2a}, kappa) and C_5Mab-8 (rat IgG_1, kappa), recognized mCCR5-overexpressing Chinese hamster ovary-K1 (CHO/mCCR5) and an endogenously mCCR5-expressing cell line (L1210) by flow cytometry. The dissociation constant (K_D) values of C_5Mab-4 and C_5Mab-8 for CHO/mCCR5 were determined as 3.5×10^{-8} M and 7.3×10^{-9} M, respectively. Furthermore, both C_5Mab-4 and C_5Mab-8 could detect mCCR5 by western blotting. These results indicated that C_5Mab-4 and C_5Mab-8 are useful for detecting mCCR5 by flow cytometry and western blotting and provide a possibility to obtain the proof of concept in preclinical studies.

Keywords: mouse CCR5, monoclonal antibody, peptide immunization, flow cytometry, western blotting

Introduction

C-C chemokine receptor 5 (CCR5) is a G protein-coupled receptor (GPCR) that plays an important role in regulating lymphocyte migration and activation.[1] CCR5 is expressed on dendritic cells, macrophages, and natural killer cells, which are cells of the innate immune response, as well as on T and B cells of the adaptive immune response.[2] CCR5 serves as a receptor for macrophage-inflammatory protein-1α (MIP-1α); also known as C-C chemokine ligand 5 (CCL5), MIP-1β; also known as C-C chemokine ligand 4 (CCL4), and inflammatory β-chemokines including regulated on activation, normal T-expressed and secreted (RANTES); also known as CCL5.[2] CCR5 binds to these ligands and activates downstream signaling, which mediates the innate and adaptive immune response through the promotion of lymphocyte migration and the secretion of proinflammatory cytokines.[3] These functions mediate the progression of tumors[4,5] and inflammatory diseases.[3]

CCR5 is identified as the principal coreceptor for human immunodeficiency virus type 1 (HIV-1),[6] and is involved in disease progression in tumors[4] and inflammatory diseases.[7] CCR5 has also been implicated in the inflammatory complications of coronavirus disease 2019 (COVID-19).[8] Therefore, a CCR5 antagonist, maraviroc, was first developed for acquired immunodeficiency syndrome (AIDS) therapy and is the only U.S. Food and Drug Administration (FDA)-approved drug for marketing.[9] Furthermore, CCR5 antagonists, including monoclonal antibodies (mAbs), have been tested in various clinical trials.[10] An anti-CCR5 mAb (PRO 140) has been developed and investigated for HIV, breast cancer, and COVID-19 in phase II or III trials.[3,11]

We have developed anti-mouse chemokine receptor mAbs against CCR1 (clone C_1Mab-6),[12] CCR3 (clones C_3Mab-2, C_3Mab-3, and C_3Mab-4),[13–15] CCR5 (clone C_5Mab-2),[16] CCR8 (clones C_8Mab-1, C_8Mab-2, and C_8Mab-3),[17–19] CXCR1 (clone Cx_1Mab-1),[20] CXCR3 (clone Cx_3Mab-4),[21] and CXCR4 (clone Cx_4Mab-1)[22] using the Cell-Based Immunization and Screening (CBIS) method. The CBIS method includes the immunization of antigen-overexpressing cells and high-throughput hybridoma screening using flow cytometry. Furthermore, we established anti-mouse chemokine receptor mAbs against CCR2 (clone

Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan.
*These authors contributed equally to this work.

C_2Mab-6),[23] CCR3 (clones C_3Mab-6 and C_3Mab-7),[24] CCR4 (clone C_4Mab-1),[25] CCR9 (clone C_9Mab-24),[26] and CXCR6 (clone Cx_6Mab-1)[27] using the N-terminal peptide immunization.

In this study, novel anti-mCCR5 mAbs were developed by the N-terminal peptide immunization.

Materials and Methods

Cell lines

The L1210 cell line (mouse lymphocytic leukemia) was obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging, and Cancer Tohoku University (Miyagi, Japan). The LN229, Chinese hamster ovary (CHO)-K1, and P3X63Ag8U.1 (P3U1) cell lines were obtained from the American Type Culture Collection (Manassas, VA).

The synthesized DNA (Eurofins Genomics KK, Tokyo, Japan) encoding mCCR5 (Accession No.: NM_009917) was subsequently subcloned into pCAGzeo and pCAGzeo-cPA vectors (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The PA tag can be detected by an anti-human podoplanin mAb (clone NZ-1).[28] The mCCR5 plasmids were transfected into CHO-K1 and LN229, using a Neon transfection system (Thermo Fisher Scientific Inc., Waltham, MA). Stable transfectants were established by cell sorting using a cell sorter (SH800; Sony Corp., Tokyo, Japan) and were cultivated in a medium containing 0.5 mg/mL of Zeocin (InvivoGen, San Diego, CA).

GeneArt™ CRISPR nuclease vectors with OFP plasmid, which target mCCR5 (TrueGuide™ Synthetic sgRNA, CRISPR59005_SGM), were purchased from Thermo Fisher Scientific Inc. The knockout plasmid was transfected into L1210 cells using a Neon transfection system. mCCR5 knockout L1210 (BINDS-57) was established by staining with anti-mCCR5 mAb (clone HM-CCR5, BioLegend, San Diego, CA) and sorted using a cell sorter (SH800).

CHO-K1, mCCR5-overexpressing CHO-K1 (CHO/mCCR5), mCCR5-PA-overexpressing CHO-K1 (CHO/mCCR5-PA), L1210, BINDS-57, and P3U1 were cultured in a (RPMI)−1640 medium (Nacalai Tesque, Inc., Kyoto, Japan) with 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific Inc.), 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B (Nacalai Tesque, Inc.). LN229 and mCCR5-overexpressing LN229 (LN229/mCCR5) were cultured in Dulbecco's modified Eagle's medium (DMEM; Nacalai Tesque, Inc.) supplemented with 10% FBS, 100 units/mL of penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. All cells were grown in a humidified incubator at 37°C, in an atmosphere of 5% CO_2 and 95% air.

Peptides

Eurofins Genomics KK (Tokyo, Japan) synthesized a partial sequence of the N-terminal extracellular region of mCCR5 ($_1$-MDFQGSVPTYSYDIDYGMS-$_{19}$) with a C-terminal cysteine. Subsequently, keyhole limpet hemocyanin (KLH) was conjugated to the C-terminus of the peptide.

Production of hybridomas

A five-week-old Sprague–Dawley rat was purchased from CLEA Japan (Tokyo, Japan). The animal was housed under specific pathogen-free conditions. All animal experiments were performed according to the relevant guidelines and regulations to minimize animal suffering and distress in the laboratory. The Animal Care and Use Committee of Tohoku University (Permit number: 2022MdA-001) approved the animal experiments. The rat was monitored daily for health during the complete four-week duration of the experiment. A reduction of more than 25% of the total body weight was defined as a humane endpoint. During the sacrifice, the rat was euthanized through cervical dislocation, after which death was verified through respiratory and cardiac arrest.

To develop mAbs against mCCR5, we intraperitoneally immunized one rat with 100 μg of the KLH-conjugated mCCR5 peptide (mCCR5-KLH) with Alhydrogel adjuvant 2% (InvivoGen). The procedure included three additional immunizations every week (100 μg/rat), followed by a final booster intraperitoneal injection (100 μg/rat) two days before the harvest of spleen cells. The harvested spleen cells were subsequently fused with P3U1 cells using PEG1500 (Roche Diagnostics, Indianapolis, IN), after which hybridomas were grown in RPMI-1640 medium with 10% FBS, 5% Briclone (NICB, Dublin, Ireland), 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B. For the hybridoma selection, hypoxanthine, aminopterin, and thymidine (HAT; Thermo Fisher Scientific Inc.) were added to the medium. The supernatants were subsequently screened using enzyme-linked immunosorbent assay (ELISA) with the mCCR5 peptide, followed by flow cytometry using CHO/mCCR5 and CHO-K1.

Antibodies

We previously developed an anti-mCCR5 mAb (C_5Mab-2) using the immunization of CHO/mCCR5 cells.[16] Alexa Fluor 488-conjugated anti-rat IgG and peroxidase-conjugated anti-rat IgG (A9542) were purchased from Cell Signaling Technology, Inc. (Danvers, MA) and Sigma-Aldrich Corp. (St. Louis, MO), respectively.

The cultured supernatants of C_5Mab-4 and C_5Mab-8-producing hybridomas were applied to 1 mL of Protein G Sepharose 4 Fast Flow (GE Healthcare, Chicago, IL) and Ab-Capcher (ProteNova, Kagawa, Japan), respectively. After washing with phosphate-buffered saline (PBS), the mAbs were eluted with an IgG elution buffer (Thermo Fisher Scientific Inc.). Finally, the eluates were concentrated, and the elution buffer was replaced with PBS using Amicon Ultra (Merck KGaA, Darmstadt, Germany).

ELISA

The synthesized mCCR5 peptide (MDFQGSVPTYSYDIDYGMSC) was immobilized on Nunc Maxisorp 96-well immunoplates (Thermo Fisher Scientific Inc.) at a concentration of 1 μg/mL for 30 minutes at 37°C. After washing with PBS containing 0.05% Tween 20 (PBST; Nacalai Tesque, Inc.), the wells were blocked with 1% bovine serum albumin (BSA)-containing PBST for 30 minutes at 37°C. Plates were then incubated with supernatants of hybridomas, followed by peroxidase-conjugated anti-rat IgG (1:20,000 diluted). Next, enzymatic reactions were conducted using the ELISA POD Substrate TMB Kit (Nacalai Tesque, Inc.), followed by measurement of the optical density at 655 nm using an iMark

microplate reader (Bio-Rad Laboratories, Inc., Berkeley, CA).

Flow cytometric analysis

Cells were harvested after a brief exposure to 0.25% trypsin and 1 mM ethylenediaminetetraacetic acid (EDTA, Nacalai Tesque, Inc.). The cells were subsequently washed with 0.1% BSA in PBS (blocking buffer) and treated with 0.01, 0.1, 1, and 10 μg/mL of primary mAbs for 30 minutes at 4°C. For the peptide inhibition assay, C_5Mab-4 and C_5Mab-8 (1 μg/mL) were preincubated with 20 μg/mL of mCCR5 peptide or dimethyl sulfoxide (DMSO) for 15 minutes at 4°C, and then incubated with the cells for 30 minutes at 4°C. The cells were treated with Alexa Fluor 488-conjugated anti-rat IgG (1:2000). The fluorescence data were collected using the SA3800 Cell Analyzer (Sony Corp.). An anti-isocitrate dehydrogenase 1 (IDH1) mAb (RcMab-1)[29,30] or an anti-mouse TERT mAb (TmMab-1, http://www.med-tohoku-antibody.com/topics/001_paper_antibody_PDIS.htm) were used as isotype control rat IgG_{2a} and rat IgG_1, respectively.

Determination of dissociation constant (K_D) by flow cytometry

CHO/mCCR5 cells were suspended in serially-diluted primary mAbs for 30 minutes at 4°C. The cells were treated with Alexa Fluor 488-conjugated anti-rat IgG (1:200). The fluorescence data were collected using the SA3800 Cell Analyzer. The K_D was subsequently calculated by fitting saturation binding curves to the built-in one-site binding models in GraphPad PRISM 6 (GraphPad Software, Inc., La Jolla, CA).

Western blotting

Cell lysates from CHO-K1 and CHO/mCCR5-PA were boiled in sodium dodecyl sulfate (SDS) sample buffer (Nacalai Tesque, Inc.). Proteins (10 μg) were separated on 5%–20% polyacrylamide gels (FUJIFILM Wako Pure Chemical Corporation) and transferred onto polyvinylidene difluoride membranes (Merck KGaA). After blocking with 4% skim milk (Nacalai Tesque, Inc.) in PBST, membranes were incubated with 1 μg/mL of C_5Mab-4, C_5Mab-8, RcMab-1, or 0.1 μg/mL of NZ-1. Membranes were then incubated with peroxidase-conjugated anti-rat IgG (diluted 1:10,000). Finally, protein bands were detected with ImmunoStar LD (FUJIFILM Wako Pure Chemical Corporation) using a Sayaca-Imager (DRC Co. Ltd., Tokyo, Japan).

Results

Development of anti-mCCR5 mAbs using N-terminal peptide immunization

To develop anti-mCCR5 mAbs, one rat was immunized with mCCR5-KLH (Fig. 1A). Spleen was then excised from the rat, after which splenocytes were fused with myeloma P3U1 cells. Developed hybridomas were subsequently seeded into fifteen 96-well plates and cultivated for six days. Then, positive wells for the naked mCCR5 peptide were selected using ELISA, followed by the selection of CHO/mCCR5-reactive and CHO-K1-nonreactive supernatants using flow cytometry (Fig. 1B). The ELISA screening identified 68 out of 1438 wells (4.7%), which strongly reacted with the naked mCCR5 peptide. The flow cytometric screening identified 18 out of the 68 wells (26.5%), which exhibited strong signals to CHO/mCCR5 cells, but not to CHO-K1 cells. After the limiting dilution and several additional screenings, C_5Mab-4 (rat IgG_{2a}, kappa) and C_5Mab-8 (rat IgG_1, kappa) were finally established (Fig. 1C).

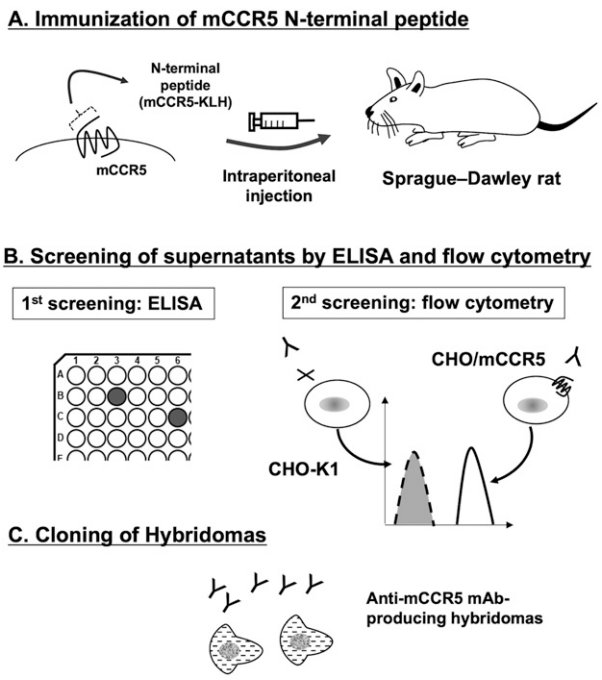

FIG. 1. The production of anti-mCCR5 mAbs. **(A)** CCR5 N-terminal peptide conjugated with KLH (mCCR5-KLH) was immunized into a Sprague–Dawley rat. The spleen cells were fused with P3U1 cells. **(B)** To select anti-mCCR5 mAb-producing hybridomas, the supernatants were screened by ELISA and flow cytometry using CHO-K1 and CHO/mCCR5 cells. **(C)** After limiting dilution, anti-mCCR5 mAbs, C_5Mab-4 and C_5Mab-8 were finally established. ELISA, enzyme-linked immunosorbent assay. mAb, monoclonal antibody.

Flow cytometric analysis using C_5Mab-4 and C_5Mab-8

We conducted flow cytometry using three anti-mCCR5 mAbs: C_5Mab-2, C_5Mab-4, and C_5Mab-8 against CHO/mCCR5 and CHO-K1 cells. C_5Mab-2 was previously established by the CBIS method.[16] Both C_5Mab-4 and C_5Mab-8 recognized CHO/mCCR5 cells dose-dependently at 10, 1, 0.1, and 0.01 μg/mL (Fig. 2A). C_5Mab-8 exhibited a superior reactivity against CHO/mCCR5 cells at 0.1 and 0.01 μg/mL compared to C_5Mab-2 and C_5Mab-4 (Fig. 2A). Parental CHO-K1 cells were not recognized by any mAbs even at 10 μg/mL (Fig. 2B). The superior reactivity of C_5Mab-8 was also observed in LN229/mCCR5 cells (Supplementary Fig. S1).

A commercially available anti-mCCR5 mAb (clone HM-CCR5) reacted with mCCR5-expressing cell line, L1210 cells (Fig. 3A). Furthermore, mCCR5-knockout L1210 (BINDS-57) cells were not recognized by HM-CCR5, indicating that L1210 cells express mCCR5 (Fig. 3A). We next investigated the reactivity of C_5Mab-4 and C_5Mab-8 against an endogenously mCCR5-expressing

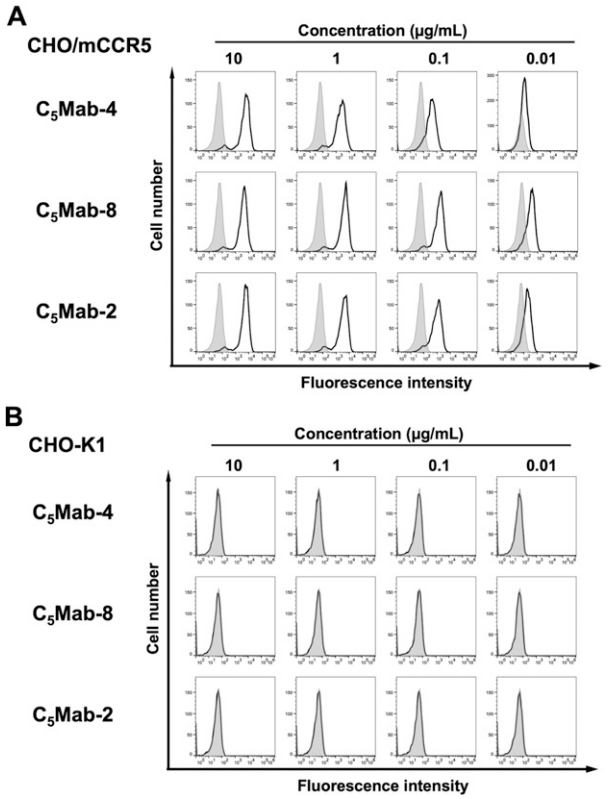

FIG. 2. Flow cytometry of mCCR5-overexpressed CHO-K1 cells using anti-mCCR5 mAbs. CHO/mCCR5 **(A)** and CHO-K1 **(B)** cells were treated with 0.01–10 μg/mL of C_5Mab-4, C_5Mab-8, or C_5Mab-2 (black line). The mAbs-treated cells were further incubated with anti-rat IgG conjugated with Alexa Fluor 488. The filled gray represents the negative control (blocking buffer). mAb, monoclonal antibody.

cell line, L1210. C_5Mab-4 reacted with L1210 at more than 1 μg/mL (Fig. 3B). C_5Mab-8 and C_5Mab-2 could react with L1210 at more than 0.1 μg/mL. We did not detect the reactivity of isotype control rat IgG_{2a} and rat IgG_1 against CHO/mCCR5 and L1210 cells (Supplementary Fig. S2).

We next performed a peptide-blocking assay. As shown in Figure 4, C_5Mab-4 and C_5Mab-8 reacted with the CHO/mCCR5 and L1210 cells. These reactions were completely neutralized by mCCR5 peptide (Fig. 4). These results indicated that the reaction by C_5Mab-4 and C_5Mab-8 was mediated by the recognition of mCCR5.

Determination of the binding affinity of C_5Mab-4 and C_5Mab-8 using flow cytometry

To determine the K_D values of C_5Mab-4 and C_5Mab-8 against CHO/mCCR5, we conducted flow cytometry. The geometric mean of the fluorescence intensity was plotted versus the concentration. The K_D values of C_5Mab-4 and C_5Mab-8 for CHO/mCCR5 were determined to be 3.5×10^{-8} M and 7.3×10^{-9} M, respectively (Fig. 5). These results indicate that C_5Mab-4 and C_5Mab-8 possess superior affinity to CHO/mCCR5 compared to C_5Mab-2 (4.3×10^{-8}).[16]

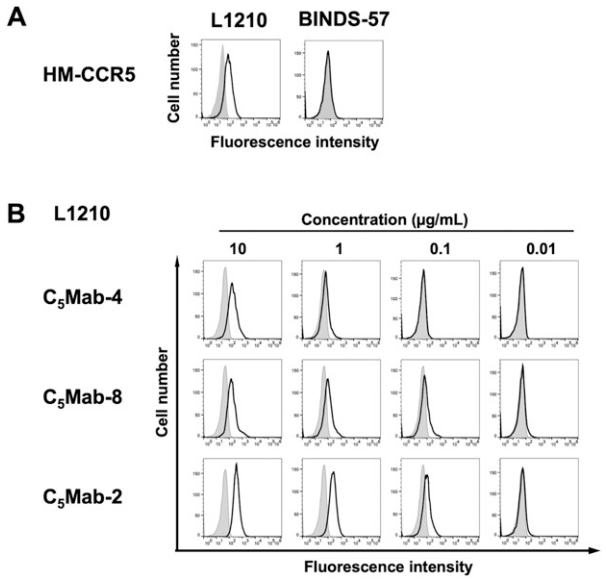

FIG. 3. Flow cytometry of endogenous mCCR5-expressing cells using anti-mCCR5 mAbs. **(A)** L1210 cells and BINDS-57 cells were treated with 1 μg/mL of HM-CCR5 (black line). The filled gray represents the negative control (blocking buffer). **(B)** L1210 cells were treated with 0.01–10 μg/mL of C_5Mab-4, C_5Mab-8, or C_5Mab-2 (black line). The mAbs-treated cells were further incubated with anti-rat IgG conjugated with Alexa Fluor 488. The filled gray represents the negative control (blocking buffer). mAb, monoclonal antibody.

Detection of mCCR5 using C_5Mab-4 and C_5Mab-8 by Western blotting

Western blotting was performed to assess the reactivity of C_5Mab-4 and C_5Mab-8. Lysates of CHO-K1 and CHO/mCCR5-PA cells were probed. C_5Mab-4 (Fig. 6A) and C_5Mab-8 (Fig. 6B) detected mCCR5 as a ~40-kDa band. In contrast, C_5Mab-4 and C_5Mab-8 did not show any bands from the lysates of CHO-K1 cells. C_5Mab-4 exhibited a superior reactivity against CHO/mCCR5 cell lysate compared to C_5Mab-8 (Fig. 6A and B, the same exposure time). In contrast, C_5Mab-2 could not detect the CHO/mCCR5 cell lysate (Fig. 6C). A similar molecular weight band was also detected by NZ-1 (Fig. 6D). An anti-IDH1 mAb, RcMab-1, was used as an internal control (Fig. 6E). These results suggest that C_5Mab-4 and C_5Mab-8 are useful for detecting mCCR5 by western blotting.

Discussion

In this study, we developed novel anti-mCCR5 mAbs (C_5Mab-4 and C_5Mab-8) using the N-terminal peptide immunization and showed their usefulness for flow cytometry (Figs. 2 and 3) and western blotting (Fig. 6) to detect mCCR5. C_5Mab-4 and C_5Mab-8 possess superior affinities (3.5×10^{-8} M and 7.3×10^{-9} M, respectively, Fig. 5) compared to that of a commercially available anti-mCCR5 mAb (clone HM-CCR5, 1.6×10^{-7} M).[16] As described in the results section, not all ELISA-positive supernatants recognized CHO/mCCR5 in flow cytometry. One possibility is a modification of the CCR5 N-terminal region. The tyrosine

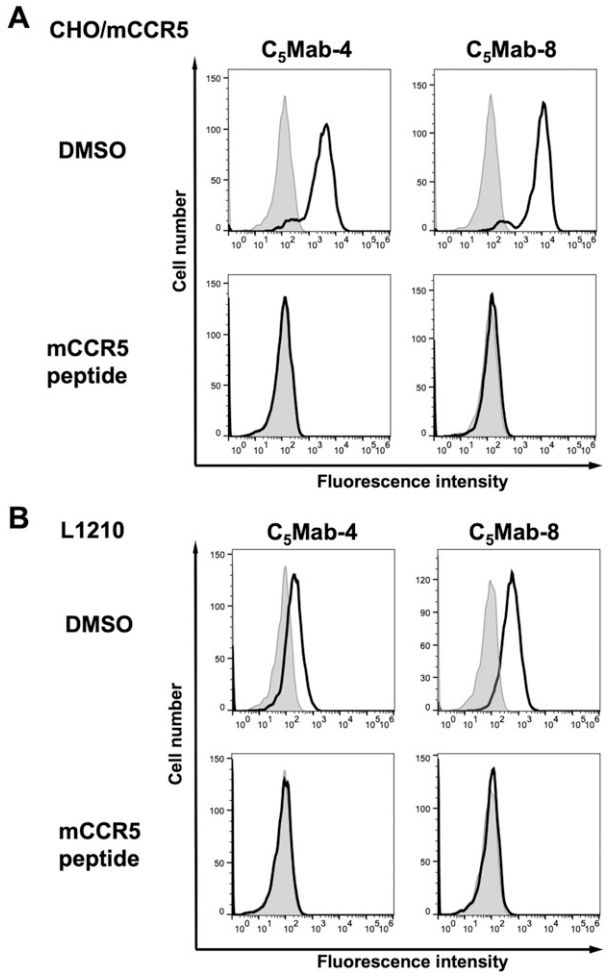

FIG. 4. A peptide-blocking assay using C_5Mab-4 and C_5Mab-8 with mCCR5 peptide. C_5Mab-4 or C_5Mab-8 (1 μg/mL) plus mCCR5 peptide (20 μg/mL) or control (2% DMSO in blocking buffer) were reacted with CHO/mCCR5 **(A)** or L1210 **(B)** for 30 minutes at 4°C, followed by treatment with Alexa Fluor 488-conjugated anti-rat IgG. The filled gray represents the negative control (blocking buffer). DMSO, dimethyl sulfoxide.

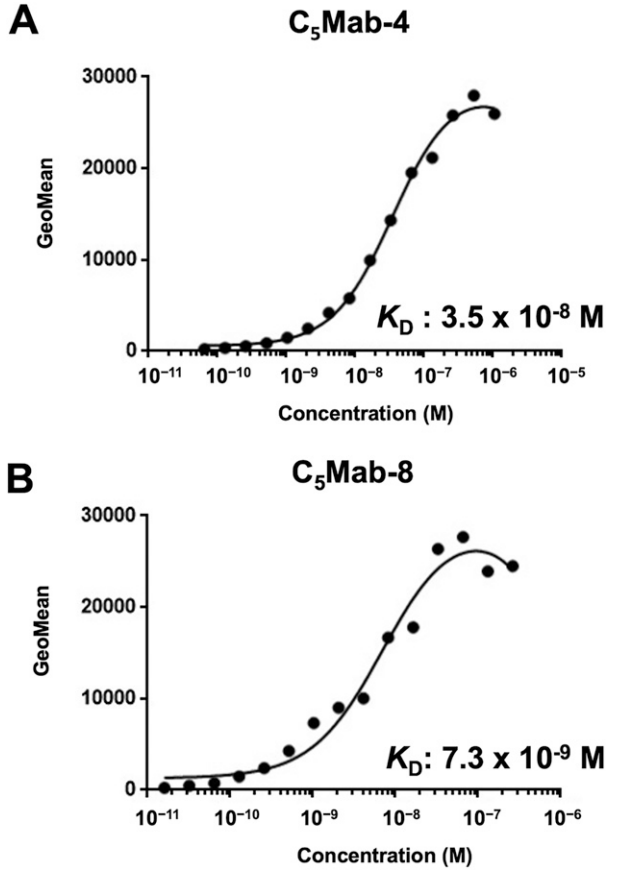

FIG. 5. The binding affinity of anti-mCCR5 mAbs. CHO/mCCR5 cells were suspended in serially diluted C_5Mab-4 **(A)** or C_5Mab-8 **(B)**. The cells were treated with anti-rat IgG conjugated with Alexa Fluor 488. The fluorescence data were subsequently collected using the SA3800 Cell Analyzer, followed by the calculation of the K_D using GraphPad PRISM 6. mAb, monoclonal antibody.

sulfation of the human CCR5 N-terminal region enhances the binding affinity of ligands and HIV envelope glycoprotein.[31,32] The human CCR5 N-terminal region possesses four potentially sulfated tyrosine residues, three of which are conserved in mCCR5 and included in the immunogen.[33] Therefore, the epitope of C_5Mab-4 and C_5Mab-8 is thought to exclude the potentially sulfated tyrosine residues. We previously determined the epitope of Cx_6Mab-1 (an anti-mouse CXCR6 mAb) using 1× and 2× alanine scanning methods.[34] We should determine the epitope C_5Mab-4 and C_5Mab-8 in future studies.

An understanding of the structure-based activation of CCR is important for the development of therapeutic agents. Among the CCR family members, human CCR5 has been structurally solved in both inactive and active states.[35,36] Based on the nuclear magnetic resonance structure of CCL5 in complex with an N-terminal fragment (residues 1 to 27) of human CCR5 sulfated at residues Y10 and Y14, the sulfation at Y10 and Y14, which are conserved in mCCR5, was also revealed to be important for chemokine affinity.[37] Furthermore, the structures of CCR8 in complex with either the antagonistic mAb or the endogenous ligand CCL1 have been determined, which provide insight into the specific activation mechanism by CCL1 and inhibition by mAb.[38] We should determine the antagonistic activity of anti-mCCR5 mAbs, which could contribute to the understanding of the inactive state of mCCR5.

Unique functions of CCR5 in the neural systems have been reported using mouse models.[39] By screening 148 transgenic and knockout mutant mouse strains for contextual memory phenotypes, CCR5 was first identified as being involved in plasticity and memory. The CCR5-knockout mice exhibited a phenotype of memory enhancements for contextual conditioning after training.[39] Furthermore, decreased CCR5 function enhances long-term potentiation and hippocampus-dependent memory, while overexpression of CCR5 in neurons caused memory deficits in mice.[39] These results indicate that CCR5 is a suppressor for cortical plasticity and hippocampus-dependent memory.

In addition, an age-related increase in CCL5–CCR5 expression in neurons leads to impairments in memory linking in middle-aged mice that can be reversed by the FDA-approved CCR5 inhibitor, maraviroc.[40] These results suggest

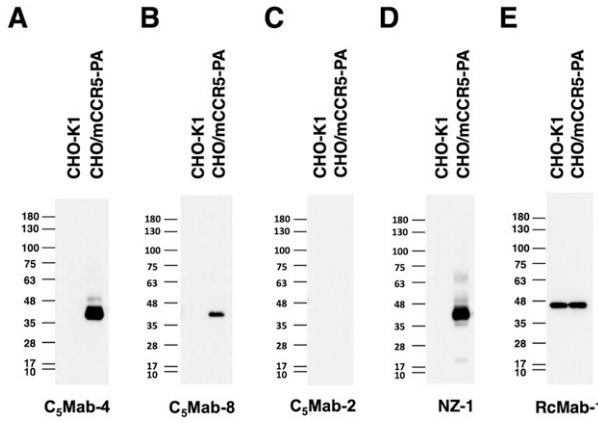

FIG. 6. Western blotting using anti-mCCR5 mAbs. The lysates of CHO-K1 and CHO/mCCR5-PA cells were electrophoresed and transferred onto polyvinylidene fluoride membranes. The membranes were incubated with 1 μg/mL of C_5Mab-4 **(A)**, 1 μg/mL of C_5Mab-8 **(B)**, 1 μg/mL of C_5Mab-2 **(C)**, 0.1 μg/mL of NZ-1 (an anti-PA tag mAb) **(D)**, or 1 μg/mL of RcMab-1 (an anti-IDH1 mAb) **(E)**. The membranes were subsequently incubated with peroxidase-conjugated anti-rat IgG. Note that the exposure time of C_5Mab-4 and C_5Mab-8 blotting was the same. IDH1, isocitrate dehydrogenase 1; mAb, monoclonal antibody.

that the CCL5–CCR5 axis is a potential target for addressing memory deficits. MAb therapies have been applied to neural disorders such as Alzheimer's disease. The accumulation of soluble and insoluble aggregated amyloid-beta (Aβ) is thought to initiate or progress pathological processes.[41] CCR5 plays an important role in modulating $A\beta_{1-42}$-induced learning and memory deficits in a mouse model, which suggests that CCR5 antagonists are a potential treatment to improve cognitive deficits associated with Alzheimer's disease.[42] Therefore, C_5Mab-4 and C_5Mab-8 could contribute to mouse preclinical studies aimed at developing treatments for memory deficits.

Authors' Contributions

R.U., H.S., T.T., and G.L. performed the experiments. M.K.K. and Y.K. designed the experiments. H.S. and M.K.K. analyzed the data. H.S. wrote the article. All authors have read and agreed to the published version of the article.

Author Disclosure Statement

The authors have no conflict of interest.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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Address correspondence to:
Yukinari Kato
Department of Antibody Drug Development
Tohoku University Graduate School of Medicine
2-1, Seiryo-machi, Aoba-ku, Sendai
Miyagi 980-8575
Japan

E-mail: yukinari.kato.e6@tohoku.ac.jp

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