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COMMUNICATION

Establishment of a high-affinity anti-mouse CXCR5 monoclonal antibody for flow cytometry

Kenichiro Ishikawa, Hiroyuki Suzuki, Tomohiro Tanaka, Mika K. Kaneko, and Yukinari Kato*

Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, 2-1, Seiryo-machi, Aoba-ku, Sendai, Miyagi, Japan

Abstract

The CXC chemokine receptor 5 (CXCR5) is a member of the G protein-coupled receptor family that is highly expressed in B cells and a subset of T cells, such as T follicular helper cells. Various types of cancers, including non-small cell lung cancer, breast cancer, and prostate cancer, also express CXCR5. Therefore, antibodies that specifically bind to CXCR5 could be useful for clarification of the mechanisms of cancer progression. In this study, we aimed to develop high-affinity monoclonal antibodies targeting mouse CXCR5 (mCXCR5) for flow cytometry. The established anti-mCXCR5 mAb (Cx_5Mab-3; rat IgG_{2b}, kappa), demonstrated reactivity with mCXCR5-overexpressed Chinese hamster ovary (CHO)-K1 (CHO/mCXCR5) in flow cytometry. Kinetic analyses using flow cytometry indicated that the dissociation constants (K_D) of Cx_5Mab-3 for CHO/mCXCR5 cell is 7.2×10^{-10} M. Furthermore, Cx_5Mab-3 did not cross-react with other mouse CC, CXC, CX3C, and XC chemokine receptors. These results indicate that Cx_5Mab-3 is useful for detecting mCXCR5 in flow cytometry with high affinity and specificity.

Keywords: Mouse CXC chemokine receptor 5; Monoclonal antibody; Peptide immunization; Flow cytometry

***Corresponding author:**
Yukinari Kato
(yukinari.kato.e6@tohoku.ac.jp)

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1. Introduction

Chemokines are classified into four different subfamilies: CC, CXC, CX3C, and XC, which depend on the position and number of cysteine residues in their N-terminus.[1-9] The chemokine receptors belong to seven-transmembrane G-protein-coupled receptor families, and trigger intracellular signal transductions through binding to the ligands.[1,8,10-19] They play fundamental roles in development, homeostasis, immune system, cell proliferation, angiogenesis, and lymphocyte differentiation.[20-39]

The CXC chemokine receptor Type 5 (CXCR5) is predominantly expressed on the surface of B cells and a subset of T cells, such as T follicular helper (Tfh) cells.[40] The CXCR5+ Tfh cells interact with germinal center B cells to promote their differentiation into plasma cells or memory B cells formation through somatic hypermutation and class-switch.[41] Therefore, CXCR5+ Tfh cells play an important role in secondary lymphoid tissue orchestration and lymphoid neogenesis in the spleen, lymph nodes, and Peyer's patches.[8,40] CXCL13 is one of the ligands of CXCR5.[42] The CXCL13/CXCR5 axis activates downstream signaling, including PI3K/Akt, MEK/ERK, and Rac

pathways, which modulates immune cells to promote lymphocyte infiltration, activation, and differentiation, thereby enhancing the antitumor immune response.[42] Furthermore, CXCR5 is also expressed in cancer cells, which makes pivotal contributions to the development and progression.[8,43-47] Therefore, monoclonal antibodies (mAbs), which specifically target CXCR5 would be useful for cancer therapy and elucidation of the disease progression.

The Cell-Based Immunization and Screening (CBIS) method includes the immunization of antigen-overexpressed cells and high-throughput hybridoma screening using flow cytometry. We have developed specific mAbs against mouse CCR1 (mCCR1; clone C_1Mab-6),[48] mouse CCR3 (mCCR3; clone C_3Mab-3),[49] mouse CCR5 (mCCR5; clone C_5Mab-2),[50] mouse CCR8 (mCCR8; clone C_8Mab-2),[51] mouse CXCR1 (mCXCR1; clone Cx_1Mab-1),[52] mouse CXCR3 (mCXCR3; clone Cx_3Mab-4),[48] and mouse CXCR4 (mCXCR4; clone Cx_4Mab-1)[48] using the CBIS method. Furthermore, we established specific mAbs against mouse CCR2 (mCCR2; clone C_2Mab-6),[53] mCCR3 (clones C_3Mab-6 and C_3Mab-7),[54] mouse CCR4 (mCCR4; clone C_4Mab-1),[55] mouse CCR6 (mCCR6; clone C_6Mab-13),[56] mouse CCR9 (mCCR9; clone C_9Mab-24),[57] and mouse CXCR6 (mCXCR6; clone Cx_6Mab-1)[58] using the N-terminal peptide immunization. In this paper, we report the successful development of a novel anti-mouse CXCR5 (mCXCR5) mAb using the N-terminal peptide immunization method.

2. Materials and methods

2.1. Plasmids, peptides, and cell lines

The synthesized DNA encoding mCXCR5 (Accession No.: NM_007551.3), mouse XCR1 (mXCR1; Accession No.: NM_011798), and mouse CX3CR1 (mCX3CR1, Accession No.: BC012653.1) were purchased from Eurofins Genomics KK (Tokyo, Japan). The complementary DNAs (cDNAs) of mouse CCR10 (mCCR10; Accession No.: NM_007721.4; Catalog No.: MR224922), and mouse CXCR2 (mCXCR2; Accession No.: NM_009909.3; Catalog No.: MR227587) were purchased from OriGene Technologies, Inc. (Rockville, MD, USA). The mCXCR5, mXCR1, and mCX3CR1 cDNAs were cloned into the pCAGzeo vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The mCCR10 cDNA was cloned into a pCAGzeo_ssnPA16 vector. The mCXCR2 cDNA was cloned into a pCMV6neo vector.

A partial sequence of the N-terminal extracellular region of mCXCR5 ($_1$-MNYPLTLDMGSITYNMDDL-$_{19}$), with a C-terminal cysteine was obtained from Eurofins Genomics KK (Tokyo, Japan). Furthermore, the keyhole limpet hemocyanin (KLH) was conjugated at the C-terminus of the peptide.

Cell lines, including P3X63Ag8U.1 (P3U1), Chinese hamster ovary (CHO)-K1, and LN229 were purchased from the American Type Culture Collection (Manassas, VA, USA). CHO-K1, P3U1, and each chemokine receptor-expressed CHO-K1 were cultured in a Roswell Park Memorial Institute (RPMI)-1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Thermo Fisher Scientific Inc., Waltham, MA, USA), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Nacalai Tesque, Inc., Kyoto, Japan). LN229 and LN229 cells expressed mCXCR5 (LN229/mCXCR5) were cultured in a Dulbecco's Modified Eagle Medium (Nacalai Tesque, Inc., Kyoto, Japan), supplemented with 10% heat-inactivated FBS (Thermo Fisher Scientific Inc., Waltham, MA, USA), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Nacalai Tesque, Inc., Kyoto, Japan).

2.2. Animals

A 5-week-old female Sprague-Dawley rat was purchased from CLEA Japan (Tokyo, Japan). There is no influence of sex on the results of the study. The animal was housed under specific pathogen-free conditions. All animal experiments were performed according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and approved by the Animal Care and Use Committee of Tohoku University (Permit number: 2022MdA-001).

2.3. Development of transfectants

The plasmids were transfected into LN229 and CHO-K1 cells using the Neon transfection system (Thermo Fisher Scientific Inc., Waltham, MA, USA). Stable transfectants were established by staining with the following mAbs: anti-mCXCR5 mAb (clone L138D7; BioLegend, San Diego, CA, USA), anti-PA tag mAb (clone NZ-1 for mCCR10), anti-mCXCR2 mAb (clone SA045E1; BioLegend, San Diego, CA, USA), anti-mXCR1 mAb (clone ZET; BioLegend, San Diego, CA, USA), and anti-mCX3CR1 mAb (clone SA011F11; BioLegend, San Diego, CA, USA). The cells were sorted using a cell sorter (SH800; Sony Corp., Tokyo, Japan). After sorting, the cells were cultured in medium supplemented with 0.5 mg/mL of Zeocin (InvivoGen, San Diego, CA, USA) or 0.5 mg/ml of G418 (Nacalai Tesque, Inc., Kyoto, Japan). These chemokine receptors-overexpressed CHO-K1 or LN229 (e.g., CHO/mCXCR5) clones were successfully established.[58]

Stable transfectants of the following chemokine receptors were previously established: CHO/mCCR1,[48]

CHO/mCCR2,[53] CHO/mCCR3,[49] CHO/PA-mCCR4,[55] CHO/mCCR5,[50] CHO/PA-mCCR6,[56] CHO/mCCR7,[59] CHO/mCCR8,[51] CHO/mCCR9,[57] CHO/mCXCR1,[52] CHO/mCXCR3,[48] CHO/mCXCR4,[48] and CHO/mCXCR6.[58] These transfectants were detected by the following mAbs: anti-CCR1 mAb (clone S15040E; BioLegend, San Diego, CA, USA), anti-CCR2 mAb (clone EPR20844; Abcam, Cambridge, MA, USA), anti-CCR3 mAb (clone J073E5; BioLegend, San Diego, CA, USA), anti-CCR5 mAb (clone C_5Mab-2[50]), anti-CCR7 mAb (clone 4B12; BioLegend, San Diego, CA, USA), anti-CCR8 mAb (clone C_8Mab-2[51]), anti-CCR9 mAb (clone CW-1.2; BioLegend, San Diego, CA, USA), anti-CXCR1 mAb (clone 1122A; R&D Systems Inc., Minneapolis, MN, USA), anti-CXCR2 mAb, anti-CXCR3 mAb (clone CXCR3-173; BioLegend, San Diego, CA, USA), anti-CXCR4 mAb (clone L276F12; BioLegend, San Diego, CA, USA), anti-CXCR6 mAb (clone SA051D1; BioLegend, San Diego, CA, USA), anti-XCR1 mAb, anti-CX3CR1 mAb, and NZ-1.

2.4. Development of mCXCR5-producing hybridomas

A rat was immunized intraperitoneally with 100 μg of KLH-conjugated mCXCR5 peptide (mCXCR5-KLH) with Alhydrogel adjuvant 2% (InvivoGen, San Diego, CA, USA). The procedure included three additional weekly injections (100 μg/rat), followed by a final booster dose (100 μg/rat) 2 days before harvesting spleen cells. The harvested spleen cells were subsequently fused with P3U1 cells, using PEG1500 (Roche Diagnostics, Indianapolis, IN, USA). The resulting hybridomas were grown in RPMI medium supplemented with 10% FBS, 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B. For hybridoma selection, hypoxanthine, aminopterin, and thymidine (Thermo Fisher Scientific Inc., Waltham, MA, USA) were added to the medium.[58] The supernatants were subsequently screened using enzyme-linked immunosorbent assay (ELISA) with the mCXCR5 peptide, followed by flow cytometry using CHO/mCXCR5 and CHO-K1 cells.

2.5. ELISA

The synthesized peptide (MNYPLTLDMGSITYNMDDLC) was immobilized onto immunoplates. After blocking with 1% bovine serum albumin (BSA)-containing 0.05% Tween20 (PBST; Nacalai Tesque, Inc., Kyoto, Japan), the immunoplates were incubated with the hybridoma supernatants, followed by peroxidase-conjugated anti-rat immunoglobulins (1:20000 dilution; Sigma-Aldrich Corp., St. Louis, MO, USA). The enzymatic reactions were determined by measuring the optical density at 655 nm using the ELISA POD Substrate TMB Kit (Nacalai Tesque, Inc., Kyoto, Japan).

2.6. Flow cytometry analysis

Cells were washed with PBS containing 0.1% BSA (blocking buffer) and treated with 10, 1, 0.1, or 0.01 μg/mL of Cx_5Mab-3 or L138D7 for 30 min at 4°C. For the peptide inhibition assay, Cx_5Mab-3 (0.1 μg/mL) or L138D7 (0.1 μg/mL) were pre-incubated with 1 μg/mL of mCXCR5 peptide or dimethyl sulfoxide for 30 min at 4°C, and further incubated with CHO/mCXCR5 for 30 min at 4°C. The cells were then treated with anti-rat immunoglobulin G (IgG) conjugated with Alexa Fluor 488 (Cell Signaling Technology, Inc., Danvers, MA, USA). Anti-mouse IgG conjugated with Alexa Fluor 488 and anti-rabbit IgG conjugated with Alexa Fluor 488 (Cell Signaling Technology, Inc., Danvers, MA, USA) were also used to detect the primary mAbs. The fluorescence data were collected using the SA3800 Cell Analyzer (Sony Corp., Tokyo, Japan). Cells were gated on the dot plot (SSC vs. FSC), and the fluorescence intensity was analyzed using FlowJo software (BD Biosciences, Franklin Lakes, NJ, USA).

2.7. Determination of dissociation constant (K_D) by flow cytometry

The K_D was determined by fitting saturation binding curves to the built-in one-site binding models in GraphPad PRISM 6 (GraphPad Software, Inc., La Jolla, CA, USA). This analysis was performed after the flow cytometry analysis of CHO/mCXCR5 cells treated with serially diluted Cx_5Mab-3 or L138D7, followed by the incubation with anti-rat IgG conjugated with Alexa Fluor 488 (1:200 dilution).

3. Results

3.1. Development of anti-mCXCR5 mAbs using N-terminal peptide immunization

To develop anti-mCXCR5 mAbs, one rat was immunized with the KLH-conjugated mCXCR5 peptide (Figure 1A). Splenocytes from the immunized rat were fused with myeloma P3U1 cells (Figure 1B). Positive wells for the naked mCXCR5 peptide were identified using ELISA; further selection was conducted using flow cytometry to identify supernatants that were reactive to CHO/mCXCR5 cells but non-reactive to CHO-K1 cells (Figure 1C). The ELISA assay identified 74 out of 1342 wells (5.5%), which strongly reacted with the naked mCXCR5 peptide. The flow cytometry analyses identified 18 out of the 74 wells (24.3%), which exhibited strong reactivity to CHO/mCXCR5 cells while showing no reactivity to CHO-K1 cells. After the limiting dilution and several additional

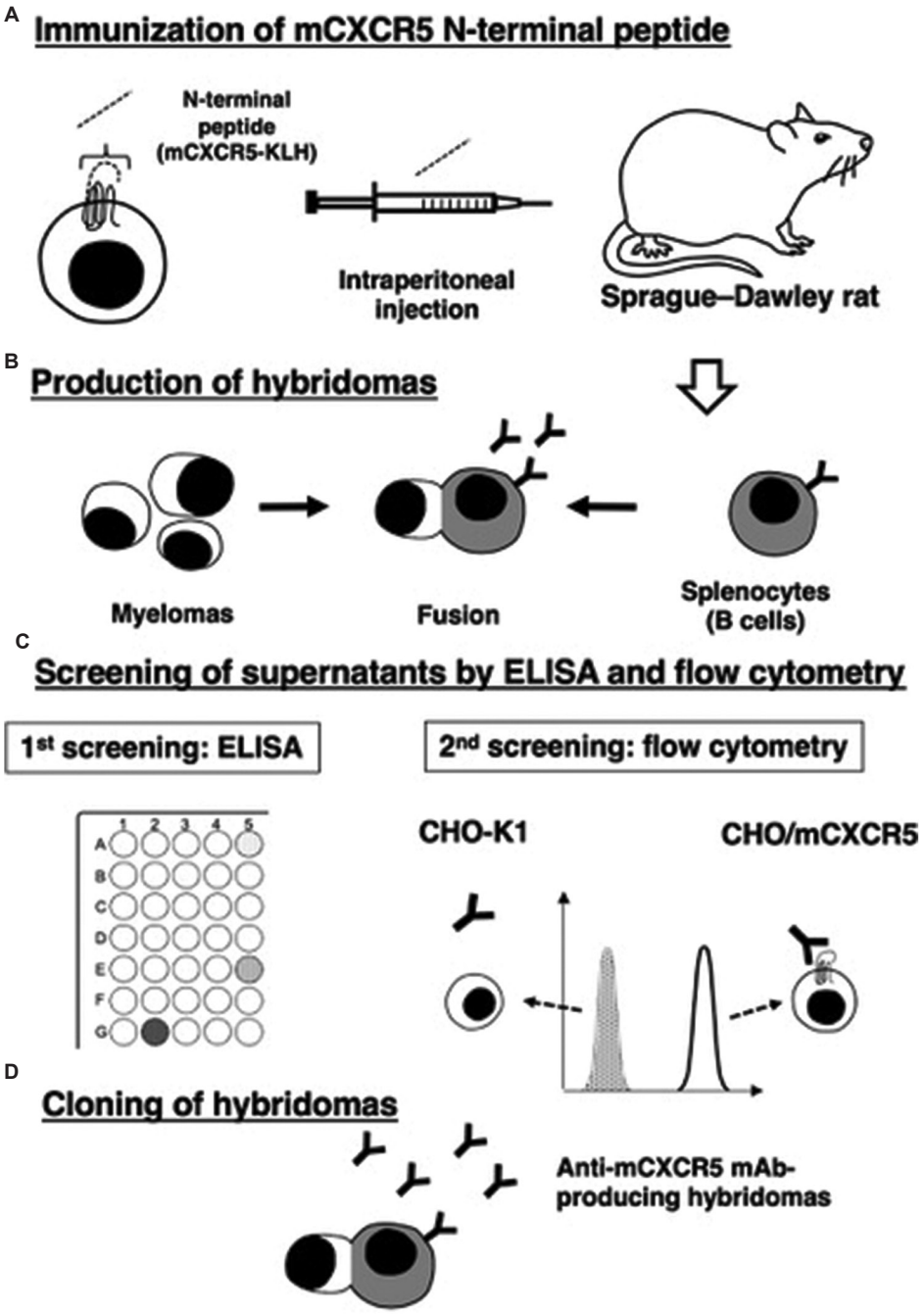

Figure 1. The development of anti-mCXCR5 mAbs. (A) Sprague-Dawley rats were immunized with CHO/mCXCR5 cells. (B) The splenocytes were fused with P3U1 myeloma cells. (C) Hybridomas producing anti-mCXCR5 mAb were screened using ELISA (first screening) and flow cytometry with CHO-K1 and CHO/mCXCR5 cells (second screening). (D) After limiting dilution, the anti-mCXCR5 mAb (Cx_5Mab-3) was successfully established.
Abbreviations: KLH: Keyhole limpet hemocyanin; CHO-K1: Chinese hamster ovary (CHO)-K1; ELISA: Enzyme-linked immunosorbent assay.

screenings, anti-mCXCR5 mAb (Cx_5Mab-3; rat IgG_{2b}, kappa) was successfully established (Figure 1D).

3.2. Flow cytometry analysis using Cx_5Mab-3

To assess the reactivity of Cx_5Mab-3 and L138D7, we conducted flow cytometry analysis on CHO/mCXCR5 and CHO-K1 cells. Cx_5Mab-3 recognized CHO/mCXCR5 cells in a dose-dependent manner at 10, 1, 0.1, and 0.01 μg/mL (Figure 2A). Parental CHO-K1 cells were not recognized even at 10 μg/mL of any mAbs (Figure 2B). The reactivity of Cx_5Mab-3 was also observed in LN229/mCXCR5 cells (Figure 3). The sensitivity against CHO/mCXCR5 or

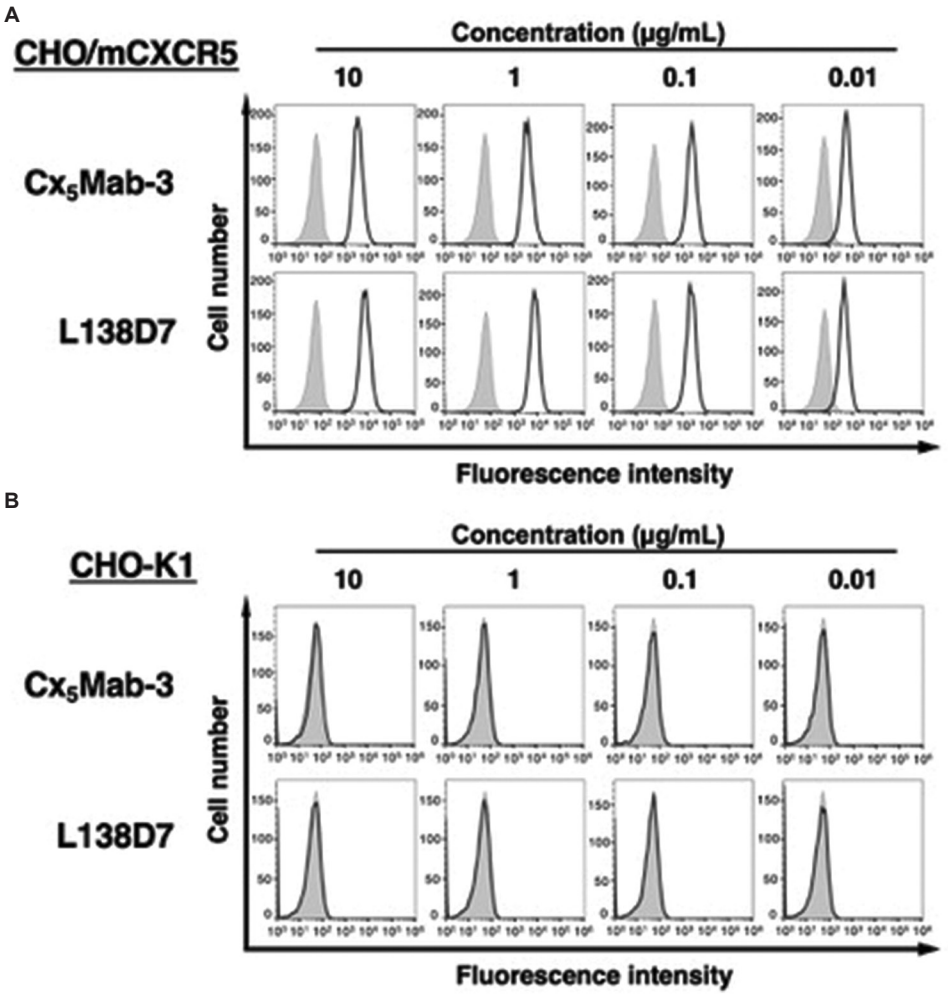

Figure 2. Flow cytometry analysis of mCXCR5-expressing cells using Cx_5Mab-3 and L138D7. CHO/mCXCR5 (A) and CHO-K1 (B) cells were treated with 0.01 – 10 μg/mL of Cx_5Mab-3 (black line), L138D7 (black line), or control blocking buffer (filled gray). Then, cells were treated with anti-rat IgG conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviations: CHO-K1: Chinese hamster ovary (CHO)-K1; IgG: Immunoglobulin G.

LN229/mCXCR5 cells was similar for both Cx_5Mab-3 and L138D7 antibodies (Figures 2 and 3).

We next performed a peptide-blocking assay. As shown in Figure 4, both Cx_5Mab-3 and L138D7 reacted with CHO/mCXCR5 cells. The reactivity of Cx_5Mab-3 was completely neutralized by the mCXCR5 peptide, indicating that its reaction was mediated by recognition of the N-terminus of mCXCR5. In contrast, the reactivity of L138D7 was not neutralized, suggesting that the epitope recognized by L138D7 differs from that recognized by Cx_5Mab-3.

3.3. Determination of dissociation constant of anti-mCXCR$_5$ mAbs against CHO/mCXCR5 cells

We determined the apparent dissociation constant (K_D) of Cx_5Mab-3 and L138D7 against mCXCR5 by flow cytometry. The geometric mean fluorescence intensity of CHO/mCXCR5 at each concentration of Cx_5Mab-3 and L138D7 was plotted. By fitting one-site binding models, the K_D values of Cx_5Mab-3 and L138D7 for CHO/mCXCR5 were determined as 7.2×10^{-10} M and 7.0×10^{-9} M (Figure 5), respectively, indicating that Cx_5Mab-3 possesses a higher affinity than L138D7 for CHO/mCXCR5 cells.

3.4. Reactivity of Cx_5Mab-3 to CC, CXC, CX3C, and XC chemokine receptor-expressed CHO-K1 cells

We have established anti-mouse CC, CXC, CX3C, and XC chemokine receptor mAbs and evaluated them using these receptors-expressed CHO-K1 cells, as described in the materials and methods. Using these eighteen cell lines, the specificity of Cx_5Mab-3 was investigated. As shown in Figure 6A, Cx_5Mab-3 recognized only CHO/mCXCR5, but

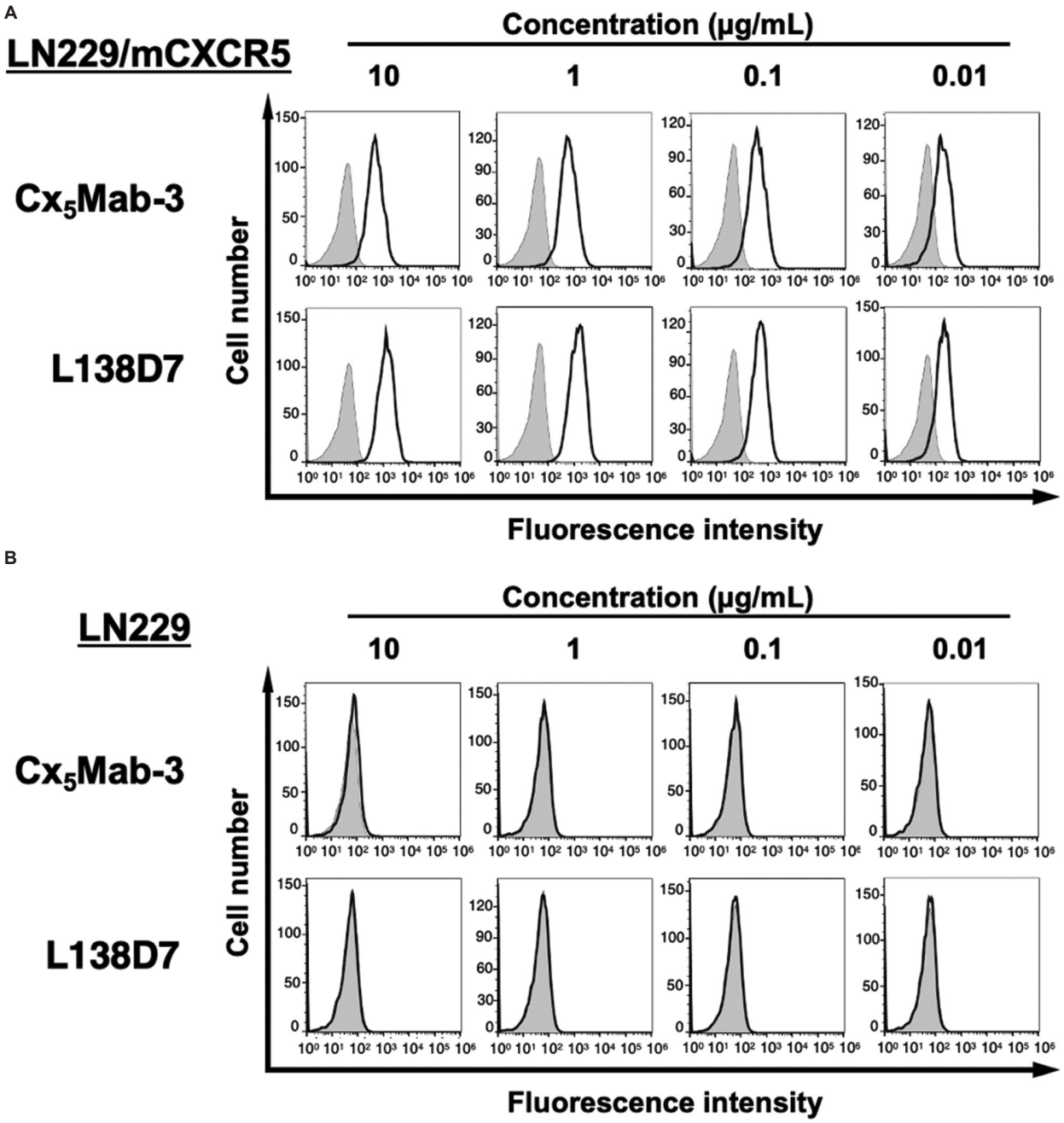

Figure 3. Flow cytometry analysis of mCXCR5-expressing LN229 cells using Cx_5Mab-3 and L138D7. LN229/mCXCR5 (A) and LN229 (B) cells were treated with 0.01 – 10 μg/mL of Cx_5Mab-3 (black line), L138D7 (black line), or control blocking buffer (filled gray). Then, cells were treated with anti-rat IgG conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviation: IgG: Immunoglobulin G.

not others. We confirmed the expression of each receptor (Figure 6B).

4. Discussion

In this study, we developed a novel anti-mCXCR5 mAb (clone Cx_5Mab-3) using N-terminal peptide immunization and investigated its application in flow cytometry to detect mCXCR5 (Figures 2-5). We confirmed the specificity of Cx_5Mab-3 among eighteen CC, CXC, CX3C, and XC chemokine receptors (Figure 6). Therefore, Cx_5Mab-3 can recognize mCXCR5-expressing cells with high specificity in *in vivo* experiments. We assessed the reactivity of

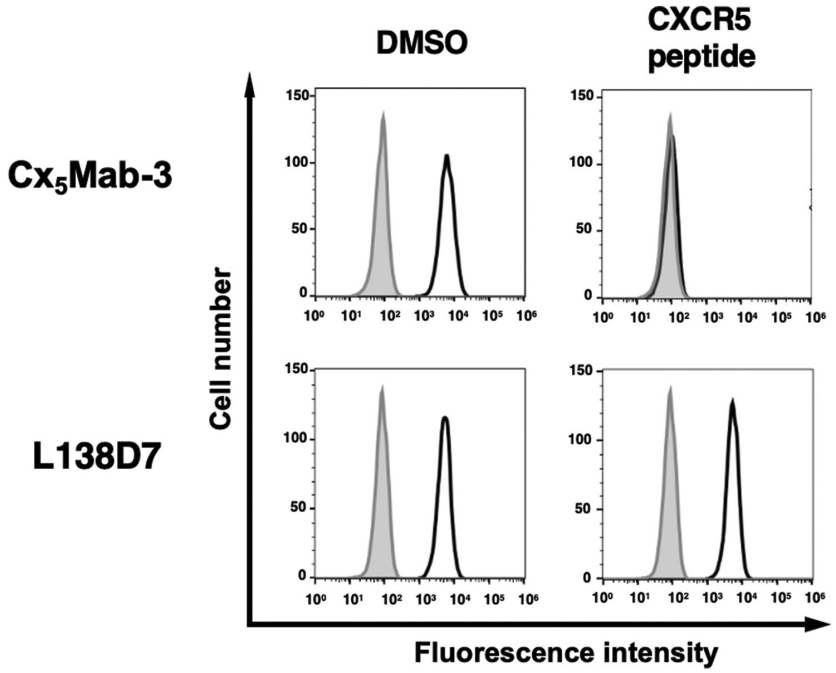

Figure 4. Peptide-blocking assay of Cx_5Mab-3 and L138D7 with mCXCR5 peptide. CHO/mCXCR5 cells were incubated with Cx_5Mab-3 (0.1 μg/mL) plus control (1% DMSO in blocking buffer), Cx_5Mab-3 plus mCCR5 peptide (1 μg/mL), L138D7 (0.1 μg/mL) plus control (1% DMSO in blocking buffer), or L138D7 plus mCCR5 peptide (1 μg/mL) for 30 min at 4°C. Cells were then treated with Alexa Fluor 488-conjugated anti-rat IgG. Fluorescence data were collected using the SA3800 Cell Analyzer. The filled gray represents the negative control (blocking buffer).
Abbreviations: DMSO: Dimethyl sulfoxide; IgG: Immunoglobulin G.

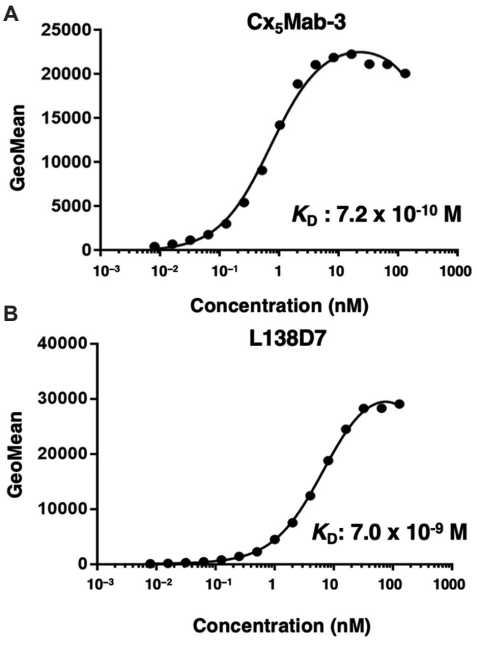

Figure 5. Binding affinity of Cx_5Mab-3 and L138D7. CHO/mCXCR5 cells were suspended in serially diluted concentrations of Cx_5Mab-3 (A) or L138D7 (B). The cells were treated with anti-rat IgG conjugated with Alexa Fluor 488. The fluorescence data were subsequently collected using the SA3800 Cell Analyzer, followed by the calculation of the K_D using GraphPad PRISM 6.
Abbreviation: IgG: Immunoglobulin G.

Cx_5Mab-3 in western blotting; however, the mCXCR5 protein was not detected by Cx_5Mab-3 (data not shown), although Cx_5Mab-3 detects N-terminal peptide of mCXCR5 in ELISA (Figure 1). This discrepancy suggests that both the cell surface-expressed N-terminal region of CXCR5 and the N-terminal peptide may form specific conformations, which are disrupted by SDS sample buffer in western blotting. A commercially available anti-mCXCR5 mAb (clone L138D7) was developed by immunizing rats with mCXCR5-transfected cells and is useful only for flow cytometry.[60] Since the reaction of L138D7 was not neutralized by the N-terminal peptide (Figure 4), its epitope may be located in other extracellular domains. In the future study, we aim to determine the binding epitope of L138D7.

It has been reported that the development of therapeutic drugs targeting the CXCL13/CXCR5 axis can be effective for treating cancers and inflammatory diseases.[61] CXCR5+ CD4+ Tfh cells mainly contribute to the antibody/B cell receptor class-switching, antibody production, and B cell proliferation during infection, autoimmunity, and cancer.[62] Moreover, CXCR5+ CD8+ T cells not only possess these functions but also maintain cytolytic activity similar to CD8+ T effector cells within tumor microenvironments.[63-65] CXCR5 expression is an important marker of progenitor memory stem-like

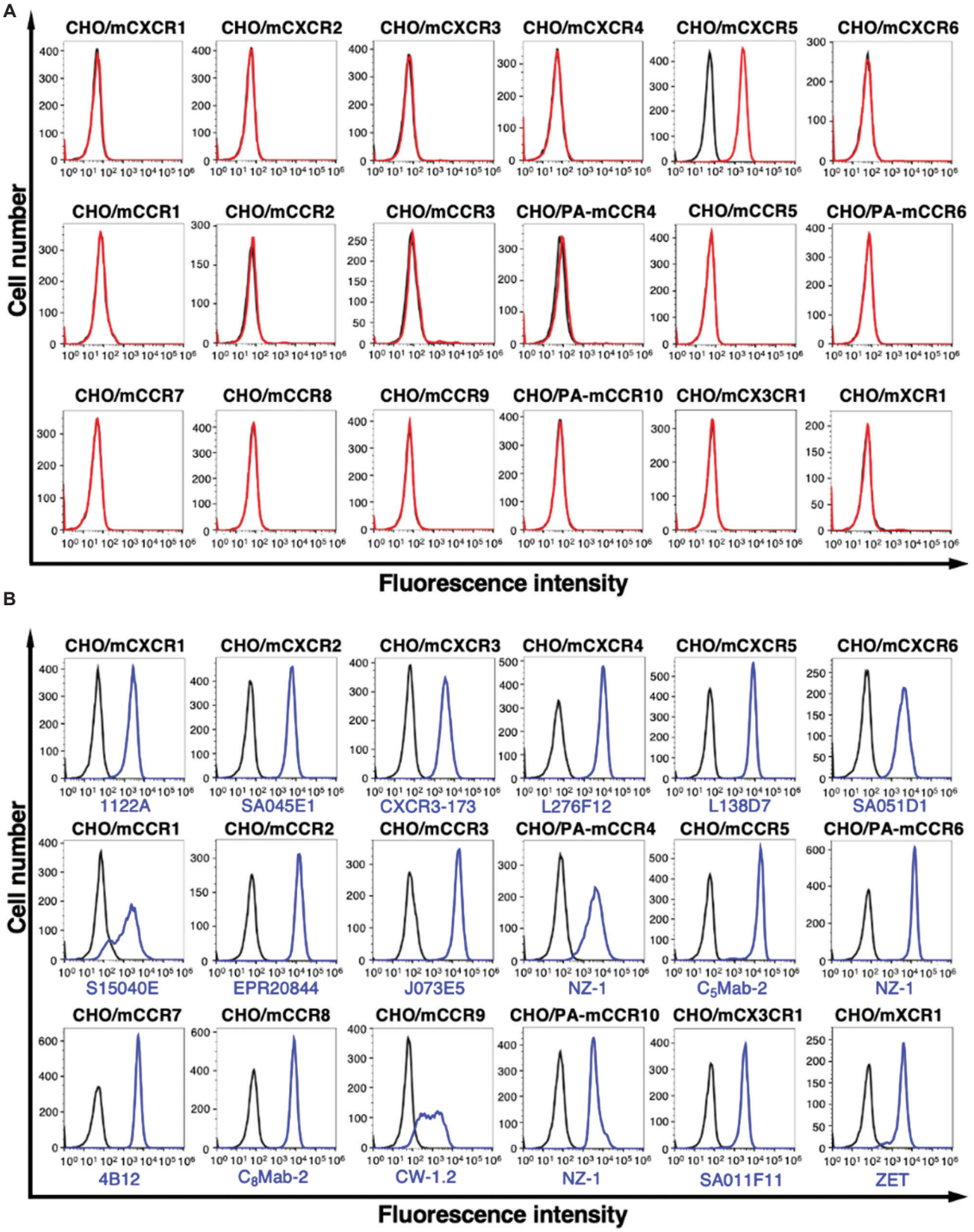

Figure 6. Flow cytometry analysis of Cx_5Mab-3 in CC, CXC, CX3C, and XC chemokine receptor-expressed CHO-K1 cells. (A) The eighteen CC, CXC, CX3C, and XC chemokine receptor-expressed CHO-K1 cells were treated with 1 μg/mL of Cx_5Mab-3 (red line) or control blocking buffer (black line), followed by the treatment with anti-rat IgG conjugated with Alexa Fluor 488. (B) The cells were treated with 1 μg/mL of corresponding mAbs, including anti-CXCR1 mAb (clone 1122A), anti-CXCR2 mAb (clone SA045E1), anti-CXCR3 mAb (clone CXCR3-173), anti-CXCR4 mAb (clone L276F12), anti-CXCR5 mAb (clone L138D7), anti-CXCR6 mAb (clone SA051D1), anti-CCR1 mAb (clone S15040E), anti-CCR2 mAb (clone EPR20844), anti-CCR3 mAb (clone J073E5), anti-CCR5 mAb (clone C_5Mab-2[50]), anti-CCR7 mAb (clone 4B12), anti-CCR8 mAb (clone C_8Mab-2[51]), anti-CCR9 mAb (clone CW-1.2), anti-XCR1 mAb (clone ZET), anti-CX3CR1 (clone SA011F11) mAb, or anti-PA tag mAb (clone NZ-1) (blue line). Then, cells were treated with corresponding secondary antibody conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviation: IgG: Immunoglobulin G.

exhausted CD8+ T cells, which can respond to immune checkpoint inhibitor therapy in tumors.[66] Furthermore, CD8+ T cells with high PD-1 expression in tumors secrete high levels of CXCL13.[67] This CXCL13 secretion attracts Tfh cells and B cells to the tumor microenvironment.[67] As a result, CXCL13 can predict the response to immune checkpoint inhibitor therapy that correlates with durable responses and increased overall survival.[67] Therefore, CXCL13 and CXCR5 are novel biomarkers for predicting responses to immune checkpoint inhibitor therapy.[68]

In human breast cancer cell lines, an inverse correlation between the p53 tumor suppressor and CXCR5 expression has been reported.[69] Silencing p53 in MCF7 cells increases CXCR5 expression, which potentiates CXCL13-mediated chemotaxis.[69] A CXCR5 promoter analysis revealed that p53 suppresses the transcriptional activity of NF-κB, which is important for the upregulation of CXCR5.[69] Similarly, related tumor suppressors p63 and p73 regulate CXCR5 through comparable mechanisms.[70] Since CXCL13 is one of the overexpressed chemokines in breast cancer tissues compared with normal breast tissues, mAb therapies targeting CXCR5 could be an important strategy in treating tumors.[70] We previously modified the isotype of mAbs to mouse IgG_{2a} to enable antibody-dependent cellular cytotoxicity (ADCC) and evaluated their antitumor activities in mouse xenograft models.[71-73] Since the isotype of Cx_5Mab-3 is rat IgG_{2b}, an isotype switch to mouse IgG_{2a} will be required in future studies.

The CXCL13/CXCR5 axis is involved in the progression of autoimmune and inflammatory diseases.[65] In inflammatory bowel disease (IBD), CXCL13 levels are elevated in both humans and mouse models.[74] In IBD patients, serum CXCL13 concentrations are significantly higher than that in healthy controls.[74] Similarly, in a mouse model of dextran sodium sulfate-induced colitis, elevated CXCL13 levels were observed in the colon. The CXCL13 deficiency inhibits the occurrence and development of the colitis and restricts CD4+CXCR5+ T cells migration to mesenteric lymph nodes, resulting in an increase of regulatory B cells in the colon.[74] Therefore, antagonizing the CXCL13/CXCR5 axis may work as a potential therapeutic strategy for patients with IBD. Cx_5Mab-3 could contribute to the preclinical study through antagonizing the CXCL13/CXCR5 axis or depleting CXCR5+ T cells. As such, Cx_5Mab-3 may be useful for establishing proof of concept in preclinical studies.[71-73]

5. Conclusion

Cx_5Mab-3 is useful for detecting mCXCR5 by flow cytometry with high affinity and may be useful for establishing proof of concept in preclinical studies.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Mika K. Kaneko, Yukinari Kato
Formal analysis: Kenichiro Ishikawa
Investigation: Kenichiro Ishikawa, Hiroyuki Suzuki, Tomohiro Tanaka
Methodology: Mika K. Kaneko
Writing–original draft: Kenichiro Ishikawa
Writing–review & editing: Hiroyuki Suzuki, Yukinari Kato

Ethics approval and consent to participate

All animal experiments were approved by the Animal Care and Use Committee of Tohoku University (Permit number: 2022MdA-001).

Consent for publication

Not applicable.

Availability of data

The data presented in this study are available in the article.

Further disclosure

The paper has been uploaded to a preprint server (doi: 10.20944/preprints202410.0497.v1).

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