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C_8Mab-3: An Anti-Mouse CCR8 Monoclonal Antibody for Immunocytochemistry

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The C-C motif chemokine receptor 8 (CCR8) is highly expressed in regulatory T cells. CCR8 is also expressed in many cancers and is associated with those progression. The development of monoclonal antibodies (mAbs) for CCR8 leads to cancer immunotherapy and elucidation of unknown mechanisms of CCR8-dependent cancer progression. In this study, we have developed an anti-mouse CCR8 (mCCR8) mAb (clone C_8Mab-3, rat IgG_1, kappa) using the Cell-Based Immunization and Screening (CBIS) method. We revealed that C_8Mab-3 and its recombinant antibody (recC_8Mab-3) bind to mCCR8-overexpressed Chinese hamster ovary (CHO)-K1 cells (CHO/mCCR8), but not to the parental CHO-K1 cells, in flow cytometry. In addition, C_8Mab-3 and recC_8Mab-3 reacted to P388 (a mouse lymphocyte-like cell) and J774-1 (a mouse macrophage-like cell), which express endogenous mCCR8. C_8Mab-3 also detected exogenous and endogenous mCCR8 using immunocytochemistry. These results suggest that C_8Mab-3, developed using the CBIS method, is useful for immunocytochemistry against exogenous and endogenous mCCR8.

Keywords: CCR8, monoclonal antibody, CBIS, flow cytometry, immunofluorescence

Introduction

The C-C motif chemokine receptor 8 (CCR8) is a family of the seven transmembrane domain G protein-coupled receptors (GPCRs) mainly expressed by regulatory T cells (Tregs).[1–5] Human CCR8 has four ligands: C-C motif chemokine ligand 1 (CCL1), CCL8, CCL16, and CCL18,[6] whereas only three of them, CCL1, CCL8, and CCL16, are expressed in mouse.[7–9] CCR8 is the only known receptor for CCL1,[5] whereas the other CCR8 ligands bind several chemokine receptors.[7–9] The $CCR8^+$ Tregs are master drivers of immune regulation. In the mice model of encephalomyelitis, a T cell-mediated autoimmune disease of the central nervous system (CNS), the number of $CCR8^+$ Tregs is reduced in the periphery. Moreover, CNS $CCR8^+$ Tregs are potentiated by CCL1, which restricts the progression of the disease.[10]

Therapeutic monoclonal antibodies (mAbs) targeting immune checkpoints have changed the therapeutic strategy of tumors.[11,12] Tumors described as ''hot'' are signs of inflammation, particularly massive infiltration and enrichment with $CD8^+$ effector T cells. The immune checkpoint inhibitors are effective in hot tumors. However, immune checkpoint inhibitors' response rate is still low owing to the lack of effector $CD8^+$ T cell infiltration or massive accumulation of Tregs that suppress their activities, which is characterized as ''cold tumors.''[13,14] Anti-mouse CCR8 (mCCR8) mAbs could be used to limit cancer growth in several cancer models.[15,16] Therefore, anti-CCR8 mAbs, mostly Tregs depleting antibodies, are currently developed for clinical use.

We have developed anti-GPCR mAbs including anti-mouse CCR3 mAb (clone C_3Mab-2),[17] anti-human CCR9 mAb (clone C_9Mab-1),[18] and anti-mCCR8 mAb (clone C_8Mab-2)[19] using the Cell-Based Immunization and Screening (CBIS) method. This study developed another anti-mCCR8 mAb (clone C_8Mab-3), and showed that C_8Mab-3 recognizes both exogenous and endogenous mCCR8 in flow cytometry and immunofluorescence.

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Materials and Methods

Cell lines and animals

P388 and J774-1 were obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging, and Cancer, Tohoku University (Miyagi, Japan). In addition, Chinese hamster ovary (CHO)-K1 and P3X63Ag8U.1 (P3U1) were obtained from the American Type Culture Collection (Manassas, VA).

Stable transfectants of CHO/mCCR8 were established in our previous study.[19] In brief, synthesized DNA (Eurofins Genomics KK, Tokyo, Japan) encoding mCCR8 (Accession No.: NM_007720.2) was subcloned into a pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). mCCR8 plasmid was transfected using a Neon transfection system (Thermo Fisher Scientific, Inc., Waltham, MA). CHO/mCCR8 cells were sorted using a cell sorter (SH800; Sony Corp., Tokyo, Japan) and cultivated in a medium containing 0.5 mg/mL of Zeocin (InvivoGen, San Diego, CA).

P388, J774-1, CHO-K1, P3U1, and CHO/mCCR8 were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B (Nacalai Tesque, Inc.). Cells were grown in a humidified incubator at 37°C with 5% CO_2 and 95% air.

A female Sprague-Dawley (SD) rat (6 weeks old) was purchased from CLEA Japan (Tokyo, Japan). The animal was housed under specific pathogen-free conditions. All animal experiments were conducted following the relevant guidelines and regulations to minimize animal suffering and distress in the laboratory.

Animal experiments were approved by the animal care and use committee of Tohoku University (Permit No.: 2019NiA-001). The rat was monitored daily for health during the 4 full weeks duration of the experiment. A reduction of >25% of the total body weight was defined as a humane endpoint. The rat was euthanized by cervical dislocation, and death was verified by respiratory and cardiac arrest.

Hybridoma production

The CBIS method was used to develop mAbs against mCCR8.[20–26] One SD rat was immunized with CHO/mCCR8 cells (1×10^9) using intraperitoneal injection together with the Imject Alum (Thermo Fisher Scientific, Inc.). The procedure included three weekly immunization followed by a final booster intraperitoneal injection administered 2 days before harvesting spleen cells. Harvested spleen cells were subsequently fused with P3U1 cells using PEG1500 (Roche Diagnostics, Indianapolis, IN). The hybridomas were grown in RPMI 1640 medium supplemented with hypoxanthine, aminopterin, and thymidine for selection (Thermo Fisher Scientific, Inc.). Culture supernatants were screened using flow cytometry.

Production of the recombinant antibody

Variable (V_H) and constant (C_H) regions of heavy chain cDNAs of C_8Mab-3 were subcloned into the pCAG-Neo vector along with variable (V_L) and constant (C_L) regions of light chain cDNAs of C_8Mab-3 into the pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation) to produce recombinant C_8Mab-3 (recC_8Mab-3). C_8Mab-3 vectors were transfected into ExpiCHO-S cells using the ExpiCHO Expression System (Thermo Fisher Scientific, Inc.). The resulting mAb (recC_8Mab-3) was purified using Ab-Capcher ExTra (ProteNova Co., Ltd, Kagawa, Japan).

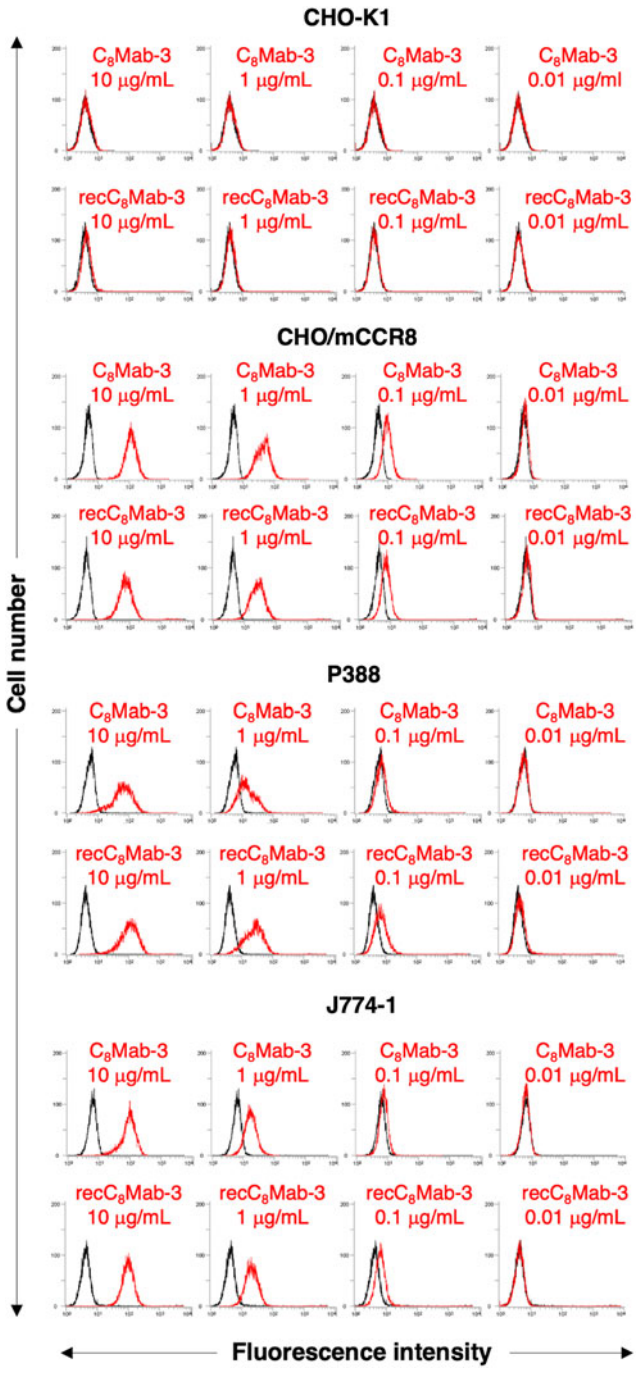

FIG. 1. Flow cytometry using anti-mCCR8 mAbs, C_8Mab-3 and recC_8Mab-3. CHO-K1, CHO/mCCR8, P388, and J774-1 were treated with 0.01–10 μg/mL C_8Mab-3 or recC_8Mab-3, followed by treatment with Alexa Fluor 488-conjugated anti-rat IgG; black line, negative control. CHO, Chinese hamster ovary; CCR8, CC motif chemokine receptor 8; mAbs, monoclonal antibodies.

Flow cytometry

We harvested cells after brief exposure to 0.25% trypsin and 1 mM of ethylenediaminetetraacetic acid (Nacalai Tesque, Inc.), washed them with 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), treated them with primary mAbs (10 μg/mL) for 30 minutes at 4°C, and treated with Alexa Fluor 488-conjugated anti-rat IgG (1:2000; Cell Signaling Technology, Inc., Danvers, MA). In addition, we collected fluorescence data using the EC800 Cell Analyzer (Sony Corp.).

Determination of binding affinity by flow cytometry

We suspended CHO/mCCR8 cells in 100 μL of serially diluted anti-mCCR8 mAbs, and then in 100 μL of Alexa Fluor 488-conjugated anti-rat IgG (1:200; Cell Signaling Technology, Inc.). Fluorescence data were collected using the EC800 Cell Analyzer. The dissociation constant (K_D) was calculated by fitting binding isotherms to built-in one-site binding models in GraphPad PRISM 8 (GraphPad Software, Inc., La Jolla, CA).

Immunocytochemistry

CHO-K1, CHO/mCCR8, P388, and J774-1 cells were cultured on acid-wash coverslips. They were treated with 4% paraformaldehyde (in PBS) for 10 minutes and in the additional 50 mM of NH_4Cl (in PBS containing 0.2 mM of Ca^{2+} and 2 mM of Mg^{2+} [PBSc/m]) for 10 minutes. Subsequently, they were incubated with a blocking buffer (PBSc/m containing 0.5% BSA) for 30 minutes, C_8Mab-3 or recC_8Mab-3 (10 μg/mL; in a blocking buffer) for 1 hour, and Alexa Fluor 488-conjugated anti-rat IgG (1:400; in a blocking buffer) for 45 minutes. 4′,6-Diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific, Inc.) was used for the cell nuclei staining. The fluorescence images were acquired on a digital microscope BZ-X800 (Keyence, Osaka, Japan) using a 40× objective.

Results

Establishment of anti-mCCR8 mAbs

We employed the CBIS method to develop anti-mCCR8 mAbs. In the CBIS method, we used stable transfectant immunization and flow cytometry-mediated hybridoma screening.[20–26] In this study, a rat was immunized with CHO/mCCR8 cells. Hybridomas were seeded into 96-well plates, and CHO/mCCR8-positive and CHO-K1-negative wells were selected. After limiting dilution, C_8Mab-3 (rat IgG_1, kappa) was finally established. We further produced its recombinant antibody (recC_8Mab-3).

Flow cytometry

We conducted flow cytometry using C_8Mab-3 and recC_8Mab-3 against CHO/mCCR8 and CHO-K1. Both C_8Mab-3 and recC_8Mab-3 reacted with CHO/mCCR8, but

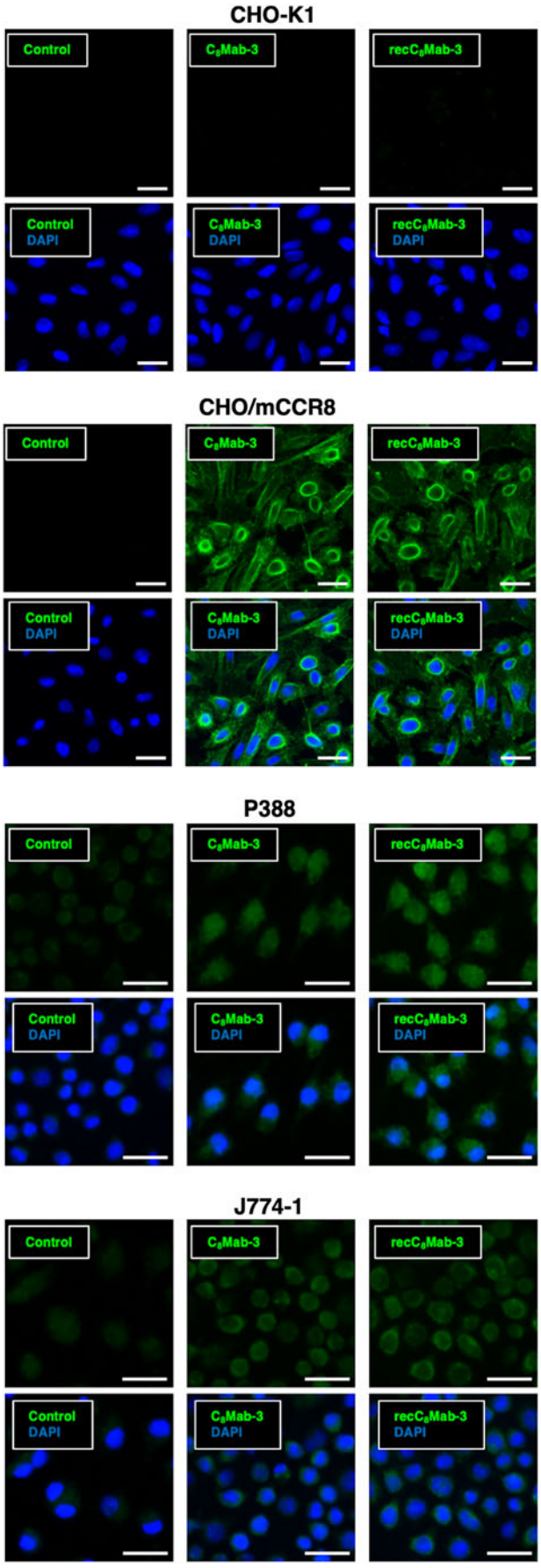

FIG. 2. Immunocytochemistry using C_8Mab-3 and recC_8Mab-3. CHO-K1, CHO/mCCR8, P388, and J774-1 were treated with buffer control, 10 μg/mL C_8Mab-3, or recC_8Mab-3, followed by treatment with Alexa Fluor 488-conjugated anti-rat IgG. DAPI was used for nuclear staining. Scale bars, 20 μm. DAPI, 4′,6-diamidino-2-phenylindole.

not with CHO-K1 (Fig. 1). In addition, both C_8Mab-3 and recC_8Mab-3 also recognized P388 and J774-1, which express endogenous mCCR8 in a dose-dependent manner (Fig. 1).

Determination of the binding affinity of C_8Mab-3 and recC_8Mab-3

The binding affinity of C_8Mab-3 and recC_8Mab-3 with CHO/mCCR8 was evaluated using flow cytometry. The K_D of C_8Mab-3 and recC_8Mab-3 for CHO/mCCR8 was 1.1×10^{-7} and 1.7×10^{-7} M, respectively (Supplementary Fig. S1), indicating that C_8Mab-3 possesses a low affinity for CHO/mCCR8 cells.

Immunocytochemical analysis using C_8Mab-3 and recC_8Mab-3

We explored the availability of C_8Mab-3 and recC_8Mab-3 in immunocytochemical analysis. First, C_8Mab-3 (10 μg/mL) and recC_8Mab-3 (10 μg/mL), but not buffer control, bound to CHO/mCCR8 (Fig. 2), whereas both antibodies did not bind to CHO-K1 (Fig. 2), suggesting that C_8Mab-3 and recC_8Mab-3 specifically detect exogenous mCCR8. Next, C_8Mab-3 and recC_8Mab-3, but not buffer control, are also bound to P388 and J774-1 cells, suggesting that both antibodies recognize endogenous mCCR8 (Fig. 2). These results show that C_8Mab-3 and recC_8Mab-3 are available in immunocytochemical analysis against exogenous and endogenous mCCR8.

Discussion

We developed a novel mAb for mCCR8, C_8Mab-3, using the CBIS method. Both C_8Mab-3 and recC_8Mab-3 bind to mCCR8 with low affinity (Supplementary Fig. S1), but they specifically recognize exogenous and endogenous mCCR8 in both flow cytometry (Fig. 1) and immunocytochemistry (Fig. 2). The confirmation to other applications, including Western blotting, immunoprecipitation, and immunohistochemistry, is required for basic study in the future.

The secretion of CCL1, a ligand of CCR8, by $CD11b^+CD14^+$ myeloid cells is involved in Treg cell infiltration.[27] Increased expression of CCR8 is observed in Treg cells and natural killer T cells, especially in cancer patients.[28] Upon binding of CCL1 to CCR8, the expression of FoxP3 is elevated through the STAT3 pathway, and the activated $CCR8^+$ Treg cells strongly suppress antitumor immunity by secretion of IL-10 and granzyme B.[10] The overall survival of breast cancer patients with high infiltration of $CCR8^+FoxP3^+$ Tregs is significantly shorter than that of patients with low infiltration.[27] Therefore, therapies that target CCL1–CCR8 molecules are desired in the clinic. Currently, we have developed three mAbs for mCCR8 (C_8Mab-1 to 3). The determination of their epitopes and neutralizing activity for CCL1–CCR8 interaction is required for *in vivo* experiments.

Tregs express several cell surface markers, including CD25, CTLA-4, PD-1, ICOS, GITR, OX40, CD15s, CCR4, and CCR8, and these markers can be used to deplete Tregs. Tregs are also indispensable for preventing autoimmunity. The removal of Tregs improves antitumor immunity and may also trigger autoimmunity.[29] Therefore, we should specifically deplete Tregs in tumors for cancer immunotherapy. Since CCR8 expression is increased in tumor-infiltrated Tregs,[28] CCR8 is one of the promising targets depleting Tregs selectively in tumors.

In our previous studies, we changed the isotype of mAbs into mouse IgG_{2a} to retain antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which caused high antitumor activities in mouse xenograft models.[30–32] Since the subclass of C_8Mab-3 is rat IgG_1, it does not possess ADCC and CDC. Therefore, in further studies, the subclass of C_8Mab-3 will be converted into mouse IgG_{2a} to assess the effect of depletion of $CCR8^+$ Treg cells in cancer immunotherapy.

In summary, we developed a novel mAb for mCCR8, C_8Mab-3, in this study. C_8Mab-3 and recC_8Mab-3 apply to flow cytometry and immunofluorescence. These mAbs would be valuable for elucidating the roles of CCR8 in cancer.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

References

1. Chensue SW, Lukacs NW, Yang TY, Shang X, Frait KA, Kunkel SL, Kung T, Wiekowski MT, Hedrick JA, Cook DN, Zingoni A, Narula SK, Zlotnik A, Barrat FJ, O'Garra A, Napolitano M, and Lira SA: Aberrant in vivo T helper type 2 cell response and impaired eosinophil recruitment in CC chemokine receptor 8 knockout mice. J Exp Med 2001; 193:573–584.
2. Freeman CM, Chiu BC, Stolberg VR, Hu J, Zeibecoglou K, Lukacs NW, Lira SA, Kunkel SL, and Chensue SW: CCR8 is expressed by antigen-elicited, IL-10-producing CD4+ CD25+ T cells, which regulate Th2-mediated granuloma formation in mice. J Immunol 2005;174:1962–1970.
3. Inngjerdingen M, Damaj B, and Maghazachi AA: Human NK cells express CC chemokine receptors 4 and 8 and respond to thymus and activation-regulated chemokine, macrophage-derived chemokine, and I-309. J Immunol 2000;164:4048–4054.
4. Inngjerdingen M, Damaj B, and Maghazachi AA: Expression and regulation of chemokine receptors in human natural killer cells. Blood 2001;97:367–375.
5. Roos RS, Loetscher M, Legler DF, Clark-Lewis I, Baggiolini M, and Moser B: Identification of CCR8, the receptor for the human CC chemokine I-309. J Biol Chem 1997;272:17251–17254.
6. Islam SA, Ling MF, Leung J, Shreffler WG, and Luster AD: Identification of human CCR8 as a CCL18 receptor. J Exp Med 2013;210:1889–1898.
7. Biber K, Zuurman MW, Homan H, and Boddeke HW: Expression of L-CCR in HEK 293 cells reveals functional responses to CCL2, CCL5, CCL7, and CCL8. J Leukoc Biol 2003;74:243–251.

8. Howard OM, Dong HF, Shirakawa AK, and Oppenheim JJ: LEC induces chemotaxis and adhesion by interacting with CCR1 and CCR8. Blood 2000;96:840–845.
9. Strasly M, Doronzo G, Cappello P, Valdembri D, Arese M, Mitola S, Moore P, Alessandri G, Giovarelli M, and Bussolino F: CCL16 activates an angiogenic program in vascular endothelial cells. Blood 2004;103:40–49.
10. Barsheshet Y, Wildbaum G, Levy E, Vitenshtein A, Akinseye C, Griggs J, Lira SA, and Karin N: CCR8(+) FOXp3(+) T(reg) cells as master drivers of immune regulation. Proc Natl Acad Sci USA 2017;114:6086–6091.
11. Kraehenbuehl L, Weng CH, Eghbali S, Wolchok JD, and Merghoub T: Enhancing immunotherapy in cancer by targeting emerging immunomodulatory pathways. Nat Rev Clin Oncol 2022;19:37–50.
12. Waldman AD, Fritz JM, and Lenardo MJ: A guide to cancer immunotherapy: From T cell basic science to clinical practice. Nat Rev Immunol 2020;20:651–668.
13. Galon J, and Bruni D: Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 2019;18:197–218.
14. Karin N: Chemokines in the landscape of cancer immunotherapy: How they and their receptors can be used to turn cold tumors into hot ones? Cancers (Basel) 2021;13:6317.
15. Eruslanov E, Stoffs T, Kim WJ, Daurkin I, Gilbert SM, Su LM, Vieweg J, Daaka Y, and Kusmartsev S: Expansion of CCR8(+) inflammatory myeloid cells in cancer patients with urothelial and renal carcinomas. Clin Cancer Res 2013;19:1670–1680.
16. Villarreal DO, L'Huillier A, Armington S, Mottershead C, Filippova EV, Coder BD, Petit RG, and Princiotta MF: Targeting CCR8 induces protective antitumor immunity and enhances vaccine-induced responses in colon cancer. Cancer Res 2018;78:5340–5348.
17. Asano T, Nanamiya R, Takei J, Nakamura T, Yanaka M, Hosono H, Tanaka T, Sano M, Kaneko MK, and Kato Y: Development of anti-mouse CC chemokine receptor 3 monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:107–112.
18. Nanamiya R, Takei J, Asano T, Tanaka T, Sano M, Nakamura T, Yanaka M, Hosono H, Kaneko MK, and Kato Y: Development of anti-human CC chemokine receptor 9 monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:101–106.
19. Tanaka T, Nanamiya R, Takei J, Nakamura T, Yanaka M, Hosono H, Sano M, Asano T, Kaneko MK, and Kato Y: Development of anti-mouse CC chemokine receptor 8 monoclonal antibodies for flow cytometry. Monoclon Antib Immunodiagn Immunother 2021;40:65–70.
20. Yamada S, Itai S, Nakamura T, Yanaka M, Kaneko MK, and Kato Y: Detection of high CD44 expression in oral cancers using the novel monoclonal antibody, C44Mab-5. Biochem Biophys Rep 2018;14:64–68.
21. Yamada S, Itai S, Nakamura T, Yanaka M, Chang YW, Suzuki H, Kaneko MK, and Kato Y: Monoclonal antibody L1Mab-13 detected human PD-L1 in lung cancers. Monoclon Antib Immunodiagn Immunother 2018;37:110–115.
22. Tanaka T, Asano T, Sano M, Takei J, Hosono H, Nanamiya R, Nakamura T, Yanaka M, Harada H, Fukui M, Suzuki H, Uchida K, Nakagawa T, Kato Y, and Kaneko MK: Development of monoclonal antibody PMab-269 against California Sea Lion Podoplanin. Monoclon Antib Immunodiagn Immunother 2021;40:124–133.
23. Kaneko MK, Sano M, Takei J, Asano T, Sayama Y, Hosono H, Kobayashi A, Konnai S, and Kato Y: Development and characterization of anti-sheep podoplanin monoclonal antibodies PMab-253 and PMab-260. Monoclon Antib Immunodiagn Immunother 2020;39:144–155.
24. Furusawa Y, Kaneko MK, and Kato Y: Establishment of C20Mab-11, a novel anti-CD20 monoclonal antibody, for the detection of B cells. Oncol Lett 2020;20:1961–1967.
25. Furusawa Y, Kaneko MK, and Kato Y: Establishment of an anti-CD20 monoclonal antibody (C20Mab-60) for immunohistochemical analyses. Monoclon Antib Immunodiagn Immunother 2020;39:112–116.
26. Itai S, Fujii Y, Nakamura T, Chang YW, Yanaka M, Saidoh N, Handa S, Suzuki H, Harada H, Yamada S, Kaneko MK, and Kato Y: Establishment of CMab-43, a sensitive and specific Anti-CD133 monoclonal antibody, for immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2017;36:231–235.
27. Plitas G, Konopacki C, Wu K, Bos PD, Morrow M, Putintseva EV, Chudakov DM, and Rudensky AY: Regulatory T cells exhibit distinct features in human breast cancer. Immunity 2016;45:1122–1134.
28. Ohue Y, and Nishikawa H: Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci 2019;110:2080–2089.
29. Tanaka A, and Sakaguchi S: Targeting Treg cells in cancer immunotherapy. Eur J Immunol 2019;49:1140–1146.
30. Takei J, Kaneko MK, Ohishi T, Hosono H, Nakamura T, Yanaka M, Sano M, Asano T, Sayama Y, Kawada M, Harada H, and Kato Y: A defucosylated antiCD44 monoclonal antibody 5mG2af exerts antitumor effects in mouse xenograft models of oral squamous cell carcinoma. Oncol Rep 2020;44:1949–1960.
31. Itai S, Ohishi T, Kaneko MK, Yamada S, Abe S, Nakamura T, Yanaka M, Chang YW, Ohba SI, Nishioka Y, Kawada M, Harada H, and Kato Y: Anti-podocalyxin antibody exerts antitumor effects via antibody-dependent cellular cytotoxicity in mouse xenograft models of oral squamous cell carcinoma. Oncotarget 2018;9:22480–22497.
32. Hosono H, Takei J, Ohishi T, Sano M, Asano T, Sayama Y, Nakamura T, Yanaka M, Kawada M, Harada H, Kaneko MK, and Kato Y: AntiEGFR monoclonal antibody 134mG2a exerts antitumor effects in mouse xenograft models of oral squamous cell carcinoma. Int J Mol Med 2020;46:1443–1452.

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