MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 41, Number 1, 2022

DOI: 10.1089/mab.2021.0044

Epitope Mapping of Rituximab Using HisMAP Method

Teizo Asano,[1] Hiroyuki Suzuki,[2] Mika K. Kaneko,[1] and Yukinari Kato[1,2]

CD20 is expressed in the B lymphocyte, and an effective target for the detection and treatment of B cell lymphomas. Therefore, CD20 has been studied as a therapeutic target of B cell lymphomas and autoimmune disorders. Specific anti-CD20 monoclonal antibodies (mAbs), such as rituximab, ofatumumab, veltuzumab, and ocaratuzumab, have been developed. Revealing the recognition mechanism of antigen by mAbs could contribute to understanding the function of mAbs and could be useful for the development of vaccine. Rituximab is a mouse-human chimeric anti-CD20 mAb, which was developed and approved for the treatment of the B cell malignancies. Hence, the binding epitope of rituximab for CD20 has been studied. Some reports show that $_{170}$-ANPS-$_{173}$, especially Ala170 and Pro172 of CD20 are important for rituximab binding. However, only phage display results showed that $_{182}$-YCYSI-$_{186}$ of CD20 is also important for rituximab binding to CD20. In this study, we tried to determine the binding epitope of rituximab for CD20 using histidine-tag insertion for epitope mapping (HisMAP) method. The results showed that two regions of CD20 ($_{169}$-PANPSE-$_{174}$ and $_{183}$-CYSIQ-$_{187}$) are important for rituximab-binding for CD20.

Keywords: CD20, rituximab, epitope mapping, monoclonal antibody, His tag

Introduction

CD20 is a transmembrane protein, which has four transmembrane domains and two extracellular loops.[1–3] CD20 functions as a calcium-permeable cation channel in the cell membrane,[4] and is related to B cell activation, differentiation, and regulation of calcium influx.[5,6] CD20 was first identified as a human B lymphocyte-specific antigen,[7] and is expressed on B cells from pre-B to mature B cell, and is also detected in many types of non-Hodgkin's lymphoma[8,9]; therefore, CD20 is an effective target for the detection and treatment of B cell lymphomas, and has been studied as a therapeutic target of B cell lymphomas and autoimmune disorders.[10–12]

Specific and sensitive monoclonal antibodies (mAbs) are critical for the diagnosis of many types of cancer. For this reason, many anti-CD20 mAbs such as rituximab, ofatumumab, veltuzumab, and ocaratuzumab have been developed. Rituximab is a mouse/human chimeric mAb, and is first Food and Drug Administration approved mAb, and used for the treatment of lymphomas, including B cell non-Hodgkin's lymphoma and B cell chronic lymphocytic leukemia.[13] The binding of rituximab to CD20 causes cell destruction through apoptosis pathway, antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC).[14,15]

The epitope determination could contribute to understanding function of mAbs and development of vaccine. Therefore, the binding region of anti-CD20 mAbs, including rituximab on CD20, has been studied using such as crystal structure, phage display, and peptide screening.[16–23] CD20 has a small and a large extracellular loop. The small loop is between 74th and 80th amino acids of CD20, and the large loop is between 141st and 187th amino acids of CD20. Many anti-CD20 mAbs, including rituximab, bind to the large extracellular loop.[16] Some studies showed that $_{170}$-ANPS-$_{173}$, especially Ala170 and Pro172 of CD20 binds to rituximab.[18,24]

Moreover, phage display results showed that rituximab binds to a discontinuous region, $_{170}$-ANPS-$_{173}$ and $_{182}$-YCYSI-$_{186}$, of CD20.[25] However, only phage display results showed the importance of $_{182}$-YCYSI-$_{186}$ for rituximab binding to CD20, and other studies have not indicated $_{182}$-YCYSI-$_{186}$ as an epitope. Therefore, we tried to determine the epitope region of

Departments of [1]Antibody Drug Development and [2]Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan.

rituximab for CD20 using a novel epitope mapping method. We previously developed a RIEDL insertion for epitope mapping (REMAP) method, which is a simple and an efficient method for linear and conformational epitopes.[26–28] In this study, we used a histidine tag (His-tag) insertion for epitope mapping (HisMAP) method to determine the binding region of CD20 for rituximab.

Materials and Methods

Plasmid preparation

DNA encoding the CD20 gene (IRAL012D02) was provided by RIKEN BRC through the National BioResource Project of MEXT, Japan.[29,30] The open reading frame of CD20 was subcloned into a pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Insertions of a 5xHis-tag (5xH*) in the extracellular region of CD20 were performed using the HotStar HiFidelity Polymerase Kit with oligonucleotides containing 5xH* insertions at the selected position. Ile141_5xH*_Lys142 (I141_5xH*_K142) was produced for instance by inserting the 5-histidine sequence between Ile141 and Lys142 of CD20. Polymerase chain reaction fragments bearing the desired mutations were inserted into the pCAG-Ble vector using the In-Fusion HD Cloning Kit (Takara Bio, Inc., Shiga, Japan).

The 5xH* insertion mutants produced were the following: I141_5xH*_K142, K142_5xH*_I143, I143_5xH*_S144, S144_5xH*_H145, H145_5xH*_F146, F146_5xH*_L147, L147_5xH*_K148, K148_5xH*_M149, M149_5xH*_E150, E150_5xH*_S151, S151_5xH*_L152, L152_5xH*_N153, N153_5xH*_F154, F154_5xH*_I155, I155_5xH*_R156, R156_5xH*_A157, A157_5xH*_H158, H158_5xH*_T159, T159_5xH*_P160, P160_5xH*_Y161, Y161_5xH*_I162, I162_5xH*_N163, N163_5xH*_I164, I164_5xH*_Y165, Y165_5xH*_N166, N166_5xH*_C167, C167_5xH*_E168, E168_5xH*_P169, P169_5xH*_A170, A170_5xH*_N171, N171_5xH*_P172, P172_5xH*_S173, S173_5xH*_E174, E174_5xH*_K175, K175_5xH*_N176, N176_5xH*_S177, S177_5xH*_P178, P178_5xH*_S179, S179_5xH*_T180, T180_5xH*_Q181, Q181_5xH*_Y182, Y182_5xH*_C183, C183_5xH*_Y184, Y184_5xH*_S185, S185_5xH*_I186, and I186_5xH*_Q187.

Cell lines

CHO/CD20 was produced in our previous study.[29,30] CD20 mutation plasmids were transfected into CHO-K1 cells using Lipofectamine LTX with Plus Reagent (Thermo Fisher Scientific, Inc., Waltham, MA). CHO-K1 cells and transfectants were cultured in 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 streptomycin, and 0.25 μg/mL amphotericin B (Nacalai Tesque, Inc.) at 37°C in a humidified atmosphere containing 5% CO_2. The transfectants were cultivated in a medium containing 0.5 mg/mL Zeocin (InvivoGen, San Diego, CA).

Antibodies

C_{20}Mab-11 was developed as previously described.[30] Rituximab was purchased from R&D Systems (Minneapolis, MN). HisMab-1 was developed as previously described.[31]

Flow cytometry

Cells were harvested after brief exposure to 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (Nacalai Tesque, Inc.). After washing with 0.1% bovine serum albumin in phosphate-buffered saline, cells were treated with primary mAbs (1 μg/mL) for 30 minutes at 4°C and subsequently with Alexa Fluor 488-conjugated anti-mouse IgG (1:1000; Cell Signaling Technology, Inc., Danvers, MA) or FITC-conjugated anti-human IgG (1:1000; Sigma-Aldrich Corp. St. Louis, MO). Fluorescence data were collected using an SA3800 (Sony Biotechnology Corp., Tokyo, Japan) for HisMAP analyses.

Results

Production of 5xHis-tag-inserted CD20 proteins

In the HisMAP method, we utilized a 5xH* and an anti-His-tag mAb (HisMab-1).[31] 5xH* was inserted between two neighboring amino acids at the extracellular region of CD20. Rituximab can bind to CD20 when 5xH* is inserted in any region independent of the rituximab epitope (Fig. 1A). In contrast, rituximab lost its reactivity to CD20 when 5xH* is inserted into the rituximab epitope region while the conformation of the rituximab epitope is disrupted by 5xH* insertion (Fig. 1B).

In this study, we employed the HisMAP method to investigate the epitope of rituximab for CD20. We

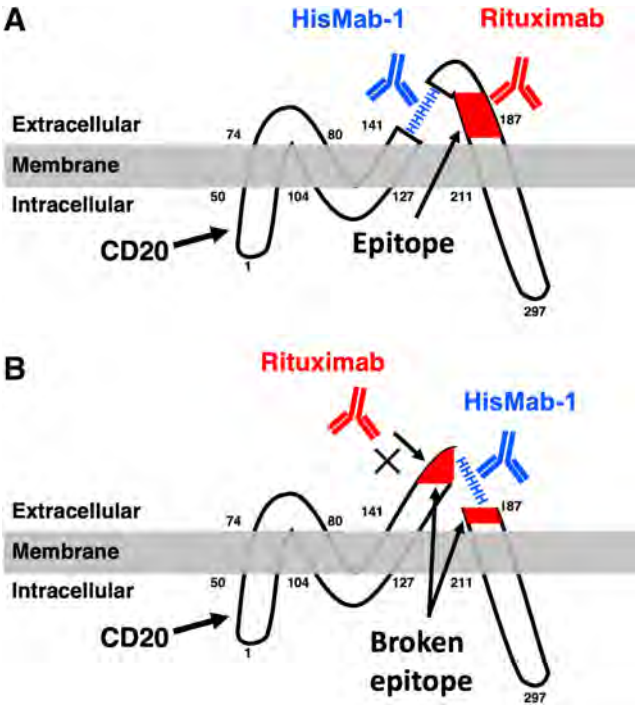

FIG. 1. Schematic illustration of HisMAP method. **(A)** The 5-histidine tag is inserted into CD20. Rituximab can bind to CD20 when 5xH* is inserted into any region, which is independent of the rituximab epitope. **(B)** Rituximab will not bind to CD20 when the conformation of the rituximab epitope is disrupted by 5xH* tag insertion. HisMAP, histidine-tag insertion for epitope mapping.

constructed 46 mutant plasmids of CD20, in which 5xH* was inserted into the second extracellular loop region of CD20 (from Ile141 to Gln187) (Fig. 2). Mutant plasmids were transfected into CHO-K1 cells, and mutant proteins were transiently expressed on CHO-K1 cells.

Determination of the rituximab-binding region using HisMAP method

Mutant protein expressing CHO-K1 cells were treated with HisMab-1 (Fig. 3A), anti-CD20 mAb (C_{20}Mab-11, Fig. 3B), or rituximab (Fig. 4), and were analyzed using flow

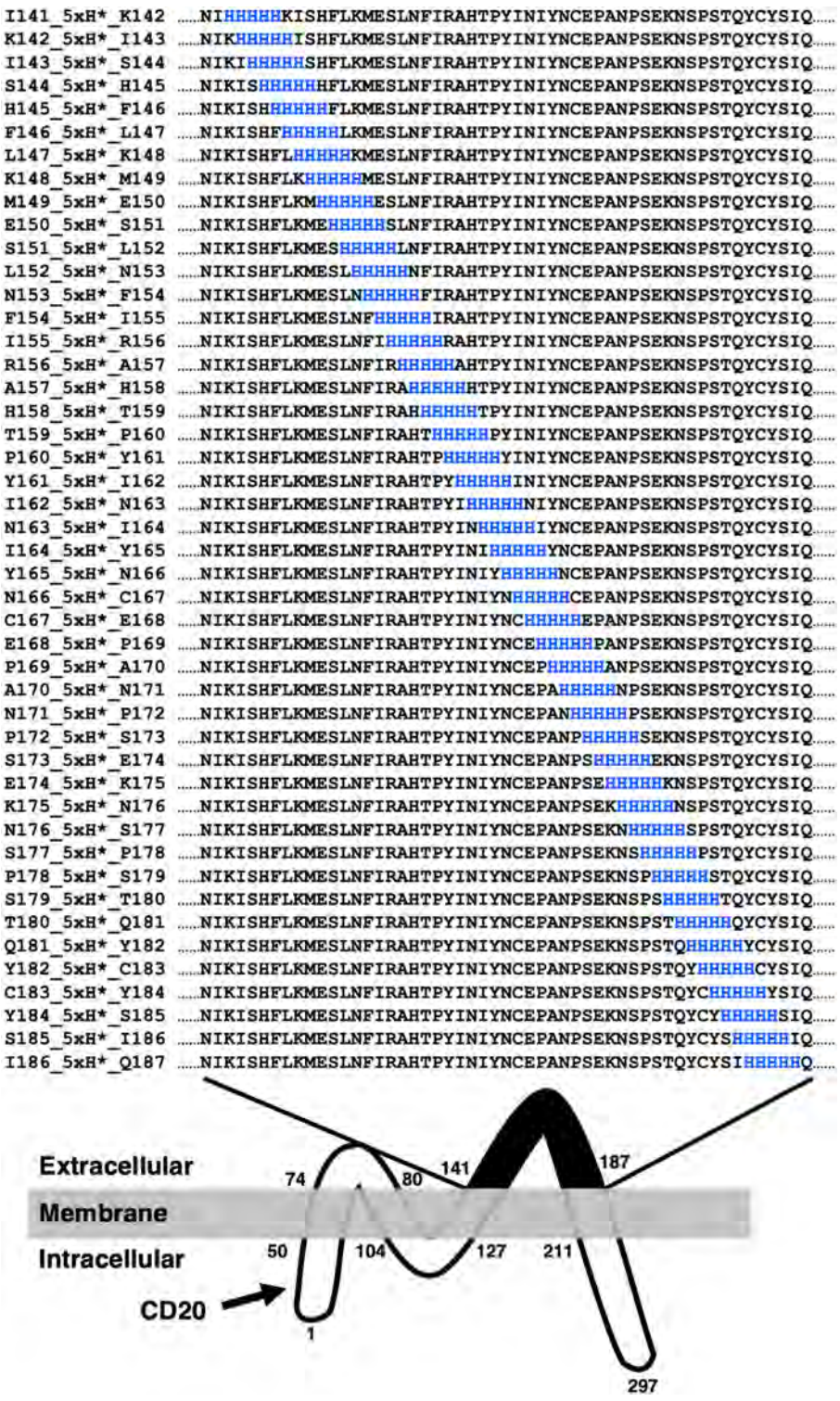

FIG. 2. Schematic illustration of histidine tag insertion mutants of CD20. The 5xH* was inserted into the large extracellular loop of CD20, and 46 mutants were constructed.

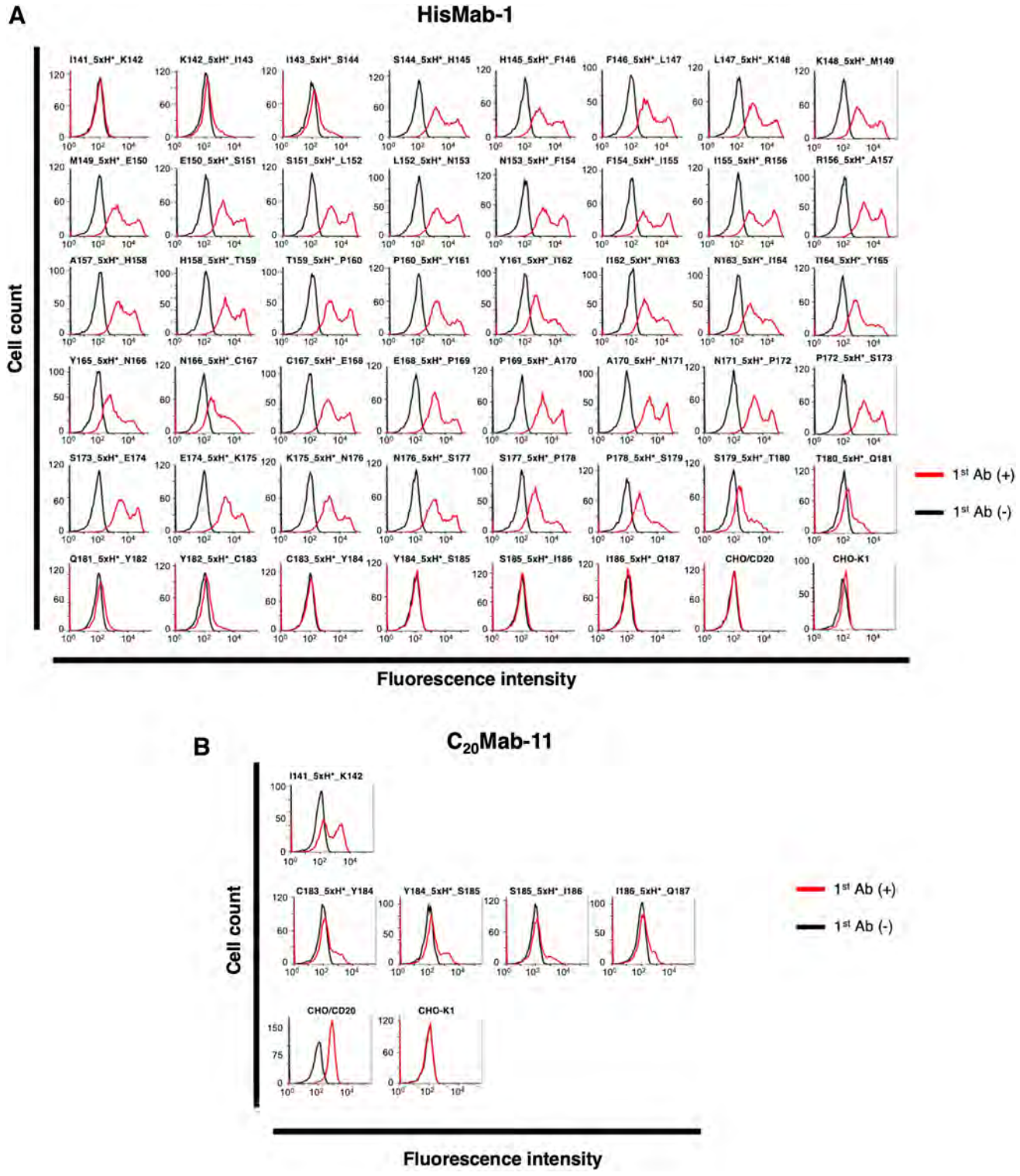

FIG. 3. Confirmation of mutant expression on CHO-K1 cells. Each 5xH* inserted mutant was expressed on CHO-K1 cells and incubated with HisMab-1 **(A)** or C_{20}Mab-11 **(B)** for 30 minutes at 4°C, followed by treatment with secondary antibody. *Red lines*: treated with HisMab-1 or C_{20}Mab-11, *black lines*: without first antibody as negative control.

cytometry. Results of the flow cytometry analysis showed that HisMab-1 used as a positive control detected 41 mutants. Although HisMab-1 did not detect five mutants (I141_5xH*_K142, C183_5xH*_Y184, Y184_5xH*_S185, S185_5xH*_I186, and I186_5xH*_Q187) (Fig. 3A), anti-CD20 mAb (C_{20}Mab-11) detected these five mutants (Fig. 3B), indicating that all CD20 mutant proteins were expressed on CHO-K1 cells. Since 5xH* was not inserted in wild-type CD20, HisMab-1 did not react with CHO/CD20 (Fig. 3A).

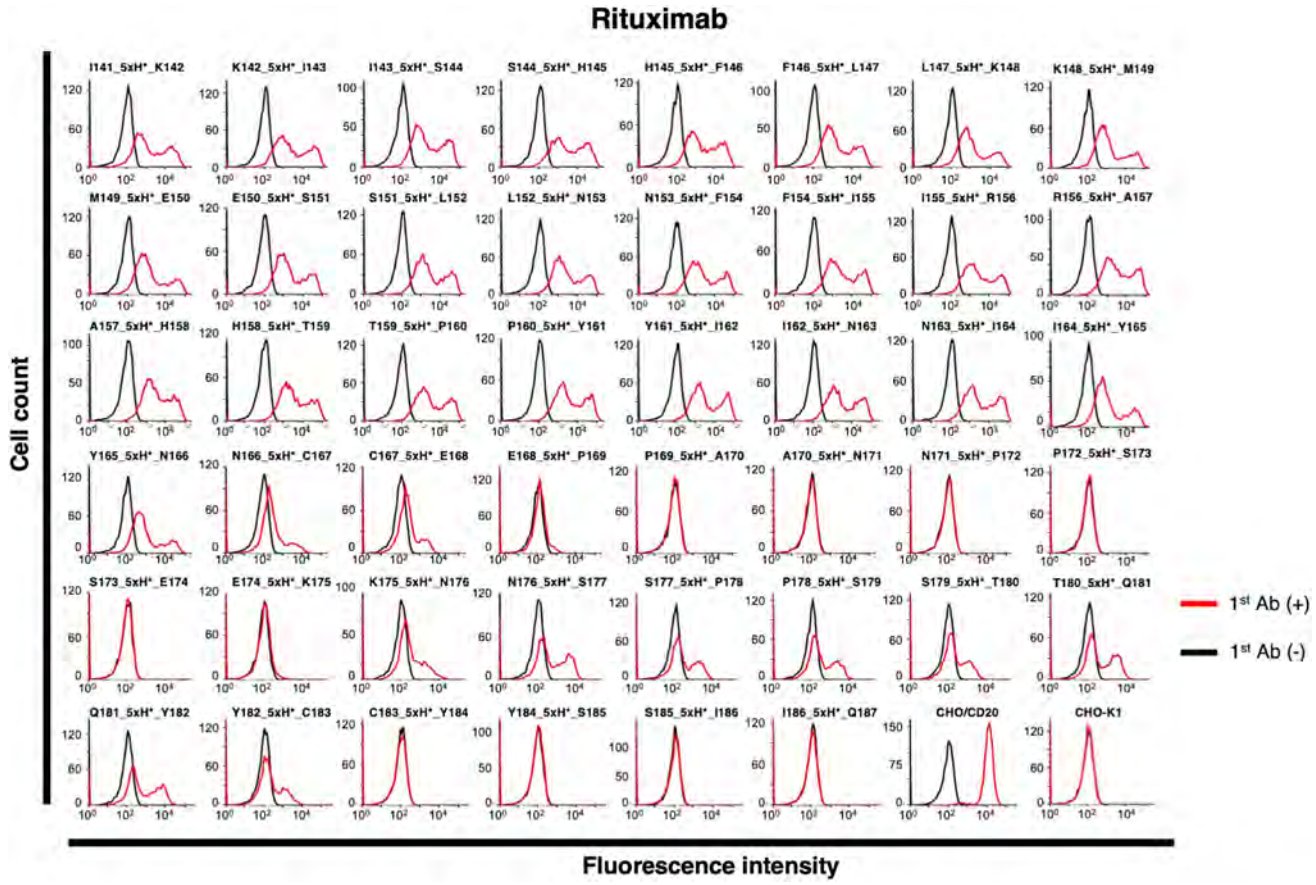

FIG. 4. Epitope mapping of rituximab using histidine tag insertion mutants of CD20. Each 5xH* inserted mutant was expressed on CHO-K1 cells and incubated with rituximab for 30 minutes at 4°C, followed by treatment with secondary antibody. *Red lines*: treated with rituximab, *black lines*: without first antibody as negative control.

In contrast, rituximab did not detect the nine mutants (P169_5xH*_A170, A170_5xH*_N171, N171_5xH*_P172, P172_5xH*_S173, S173_5xH*_E174, C183_5xH*_Y184, Y184_5xH*_S185, S185_5xH*_I186, and I186_5xH*_Q187), and weakly detected one mutant (E174_5xH*_K175) (Fig. 4).

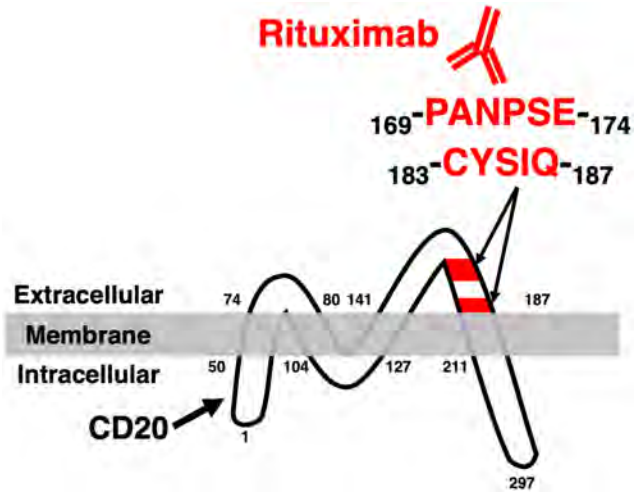

FIG. 5. Schematic illustration of epitope mapping of rituximab. Two regions ($_{169}$-PANPSE-$_{174}$ and $_{183}$-CYSIQ-$_{187}$) are important for the binding of rituximab to CD20.

These results indicate that two peptides ($_{169}$-PANPSE-$_{174}$, and $_{183}$-CYSIQ-$_{187}$) are important for binding rituximab to CD20. These results are summarized in Figure 5.

Discussion

The binding of rituximab to CD20 causes cell destruction through apoptosis pathway, ADCC, and CDC. To understand the molecular mechanism of recognition of CD20 by rituximab, the binding epitope of rituximab has been studied. Polyak et al. conducted flow cytometric analysis using point mutation of CD20.[32] Mutation of Ala170 and Pro172 in human CD20 abrogated the binding of rituximab. Although rituximab did not recognize murine CD20, introduction of Ala170 and Pro172 into the equivalent positions in murine CD20 reconstituted the epitope recognized by rituximab. These results indicated that Ala170 and Pro172 are essential for rituximab binding to CD20. Teeling et al. also reported that amino acids from Tyr165 to Tyr182 of CD20 contribute to binding of rituximab, especially Ala170 and Pro172 are absolutely essential.[18]

Binder et al. demonstrated the epitope analysis of rituximab using phage display, and results showed that rituximab binds to a discontinuous region in CD20: $_{170}$-ANPS-$_{173}$ and $_{182}$-YCYSI-$_{186}$ of CD20.[25] Moreover, the crystal structure of the rituximab Fab in complex with a synthesized peptide were solved by Du et al.[24] The $_{170}$-ANPS-$_{173}$ of CD20 is

embedded into the pocket on the surface of rituximab, and plays an essential role in the binding of rituximab to CD20. These studies showed that $_{170}$-ANPS-$_{173}$ of CD20 is important for rituximab binding. Especially, Ala170 and Pro172 of CD20 are critical epitope of rituximab. In this study, we showed that $_{169}$-PANPSE-$_{174}$ and $_{183}$-CYSIQ-$_{187}$ are important for binding rituximab to CD20 using HisMAP method. The result that $_{169}$-PANPSE-$_{174}$ peptide is important for rituximab binding is consistent with previous studies even though Pro169 and Glu174 are added.

In HisMAP method, two mutants, P169_5xH*_A170 and S173_5xH*_E174, lost their reactivity to rituximab (Fig. 4); therefore, we judged that Pro169 and Glu174 of CD20 are also important for rituximab binding. Crystal structure analysis showed that a hydrogen bonding is formed between Pro169 of CD20 and Ser50 of rituximab heavy chain. In addition to hydrogen bonding interaction, Pro169 of CD20 makes van der Waals contacts to Asn93 of rituximab light chain and Ser59 of rituximab heavy chain.[24] Glu174 of CD20 also makes van der Waals contacts to Tyr102 and Trp106 of rituximab heavy chain. Therefore, Pro169 and Glu174 also contribute to the interaction of CD20 with rituximab.

Moreover, our results indicated that $_{183}$-CYSIQ-$_{187}$ are also important for rituximab binding (Figs. 4 and 5). Binder et al. showed almost the same region as a rituximab binding motif.[25] However, crystal structure analysis showed this region is not binding to rituximab.[24] The large extracellular loop of CD20 has a disulfide bond between Cys167 and Cys183. Reduction of CD20 caused abrogation of rituximab binding to CD20.[3] This result indicated that Cys167-Cys183 disulfide bond is essential for rituximab binding to CD20, and CD20 recognition by rituximab is critically dependent on the conformation of CD20. Two epitope regions, $_{169}$-PANPSE-$_{174}$, and $_{183}$-CYSIQ-$_{187}$, are located on adjacent site because Cys167 and Cys183 make the disulfide bond. Insertion of 5xH* into $_{183}$-CYSIQ-$_{187}$ region may affect the structure of $_{169}$-PANPSE-$_{174}$ region, or may cause the disruption of Cys167-Cys183 disulfide bond resulting in inhibition of the rituximab binding.

In HisMAP method, His-tag system consists of His-tag and HisMab-1 can be used as control for expression of mutant proteins; therefore, HisMAP method is applicable to the epitope mapping for proteins for which there are not specific antibodies. However, five mutants (I141_5xH*_K142, C183_5xH*_Y184, Y184_5xH*_S185, S185_5xH*_I186, and I186_5xH*_Q187) did not react with HisMab-1. The inserted His-tag in I141_5xH*_K142 and I186_5xH*_Q187 is located on juxtamembrane; therefore, HisMab-1 may not access to the His-tag. Moreover, full-length protein expressed on membrane is used in HisMAP method, whereas a partial sequence of protein in solution is usually used in X-ray crystallography and peptide scanning; hence, HisMAP method can detect antigen–antibody interaction, which is close to native state. In this report, we showed that HisMAP method is useful for epitope mapping of rituximab; however, further studies are required for the investigation of structure analysis of interaction between HisMab-1 and inserted His-tag.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This research was supported in part by Japan Agency for Medical Research and Development (AMED) under grant nos. JP21am0401013 (to Y.K.) and JP21am0101078 (to Y.K.), and by the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (KAKENHI) grant nos. 21K15523 (to T.A.), 21K07168 (to M.K.K.), and 19K07705 (to Y.K.).

References

1. Tedder TF, Streuli M, Schlossman SF, and Saito H: Isolation and structure of a cDNA encoding the B1 (CD20) cell-surface antigen of human B lymphocytes. Proc Natl Acad Sci U S A 1988;85:208–212.
2. Einfeld DA, Brown JP, Valentine MA, Clark EA, and Ledbetter JA: Molecular cloning of the human B cell CD20 receptor predicts a hydrophobic protein with multiple transmembrane domains. EMBO J 1988;7:711–717.
3. Ernst JA, Li H, Kim HS, Nakamura GR, Yansura DG, and Vandlen RL: Isolation and characterization of the B-cell marker CD20. Biochemistry 2005;44:15150–15158.
4. Bubien JK, Zhou LJ, Bell PD, Frizzell RA, and Tedder TF: Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca2+ conductance found constitutively in B lymphocytes. J Cell Biol 1993;121:1121–1132.
5. Kuijpers TW, Bende RJ, Baars PA, Grummels A, Derks IA, Dolman KM, Beaumont T, Tedder TF, van Noesel CJ, Eldering E, and van Lier RA: CD20 deficiency in humans results in impaired T cell-independent antibody responses. J Clin Invest 2010;120:214–222.
6. Tedder TF, and Engel P: CD20: A regulator of cell-cycle progression of B lymphocytes. Immunol Today 1994;15: 450–454.
7. Stashenko P, Nadler LM, Hardy R, and Schlossman SF: Characterization of a human B lymphocyte-specific antigen. J Immunol 1980;125:1678–1685.
8. Polyak MJ, Li H, Shariat N, and Deans JP: CD20 homooligomers physically associate with the B cell antigen receptor. Dissociation upon receptor engagement and recruitment of phosphoproteins and calmodulin-binding proteins. J Biol Chem 2008;283:18545–18552.
9. Li H, Ayer LM, Lytton J, and Deans JP: Store-operated cation entry mediated by CD20 in membrane rafts. J Biol Chem 2003;278:42427–42434.
10. Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P, and Thomas ED: Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood 1987;69:584–591.
11. Smith MR: Rituximab (monoclonal anti-CD20 antibody): Mechanisms of action and resistance. Oncogene 2003;22: 7359–7368.
12. Pescovitz MD: Rituximab, an anti-cd20 monoclonal antibody: History and mechanism of action. Am J Transplant 2006;6:859–866.
13. Keating GM: Rituximab: A review of its use in chronic lymphocytic leukaemia, low-grade or follicular lymphoma and diffuse large B-cell lymphoma. Drugs 2010;70:1445–1476.
14. Glennie MJ, French RR, Cragg MS, and Taylor RP: Mechanisms of killing by anti-CD20 monoclonal antibodies. Mol Immunol 2007;44:3823–3837.
15. Lim SH, Beers SA, French RR, Johnson PW, Glennie MJ, and Cragg MS: Anti-CD20 monoclonal antibodies: Historical and future perspectives. Haematologica 2010;95:135–143.

16. Klein C, Lammens A, Schäfer W, Georges G, Schwaiger M, Mössner E, Hopfner KP, Umaña P, and Niederfellner G: Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. MAbs 2013;5:22–33.
17. Niederfellner G, Lammens A, Mundigl O, Georges GJ, Schaefer W, Schwaiger M, Franke A, Wiechmann K, Jenewein S, Slootstra JW, Timmerman P, Brännström A, Lindstrom F, Mössner E, Umana P, Hopfner KP, and Klein C: Epitope characterization and crystal structure of GA101 provide insights into the molecular basis for type I/II distinction of CD20 antibodies. Blood 2011;118:358–367.
18. Teeling JL, Mackus WJ, Wiegman LJ, van den Brakel JH, Beers SA, French RR, van Meerten T, Ebeling S, Vink T, Slootstra JW, Parren PW, Glennie MJ, van and de Winkel JG: The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 2006;177:362–371.
19. Du J, Wang H, Zhong C, Peng B, Zhang M, Li B, Hou S, Guo Y, and Ding J: Crystal structure of chimeric antibody C2H7 Fab in complex with a CD20 peptide. Mol Immunol 2008;45:2861–2868.
20. Gordon LI, Molina A, Witzig T, Emmanouilides C, Raubtischek A, Darif M, Schilder RJ, Wiseman G, and White CA: Durable responses after ibritumomab tiuxetan radioimmunotherapy for CD20+ B-cell lymphoma: Long-term follow-up of a phase 1/2 study. Blood 2004;103:4429–4431.
21. Uchiyama S, Suzuki Y, Otake K, Yokoyama M, Ohta M, Aikawa S, Komatsu M, Sawada T, Kagami Y, Morishima Y, and Fukui K: Development of novel humanized anti-CD20 antibodies based on affinity constant and epitope. Cancer Sci 2010;101:201–209.
22. Stein R, Qu Z, Chen S, Rosario A, Shi V, Hayes M, Horak ID, Hansen HJ, and Goldenberg DM: Characterization of a new humanized anti-CD20 monoclonal antibody, IMMU-106, and Its use in combination with the humanized anti-CD22 antibody, epratuzumab, for the therapy of non-Hodgkin's lymphoma. Clin Cancer Res 2004;10:2868–2878.
23. Forero-Torres A, de Vos S, Pohlman BL, Pashkevich M, Cronier DM, Dang NH, Carpenter SP, Allan BW, Nelson JG, Slapak CA, Smith MR, Link BK, Wooldridge JE, and Ganjoo KN: Results of a phase 1 study of AME-133v (LY2469298), an Fc-engineered humanized monoclonal anti-CD20 antibody, in FcγRIIIa-genotyped patients with previously treated follicular lymphoma. Clin Cancer Res 2012;18:1395–1403.
24. Du J, Wang H, Zhong C, Peng B, Zhang M, Li B, Huo S, Guo Y, and Ding J: Structural basis for recognition of CD20 by therapeutic antibody Rituximab. J Biol Chem 2007;282:15073–15080.
25. Binder M, Otto F, Mertelsmann R, Veelken H, and Trepel M: The epitope recognized by rituximab. Blood 2006;108: 1975–1978.
26. Asano T, Kaneko MK, and Kato Y: RIEDL tag: A novel pentapeptide tagging system for transmembrane protein purification. Biochem Biophys Rep 2020;23:100780.
27. Asano T, Kaneko MK, Takei J, Tateyama N, and Kato Y: Epitope mapping of the anti-CD44 monoclonal antibody (C44Mab-46) using the REMAP method. Monoclon Antib Immunodiagn Immunother 2021;40:156–161.
28. Sano M, Kaneko MK, Aasano T, and Kato Y: Epitope mapping of an antihuman EGFR monoclonal antibody (EMab-134) using the REMAP method. Monoclon Antib Immunodiagn Immunother 2021;40:191–195.
29. Furusawa Y, Kaneko MK, and Kato Y: Establishment of an anti-CD20 monoclonal antibody (C(20)Mab-60) for immunohistochemical analyses. Monoclon Antib Immunodiagn Immunother 2020;39:112–116.
30. Furusawa Y, Kaneko MK, and Kato Y: Establishment of C(20)Mab-11, a novel anti-CD20 monoclonal antibody, for the detection of B cells. Oncol Lett 2020;20:1961–1967.
31. Asano T, Takei J, Furusawa Y, Saito M, Suzuki H, Kaneko MK, and Kato Y: Epitope mapping of an anti-CD20 monoclonal antibody (C20Mab-60) using the HisMAP method. Monoclon Antib Immunodiagn Immunother 2021;40:243–249.
32. Polyak MJ, and Deans JP: Alanine-170 and proline-172 are critical determinants for extracellular CD20 epitopes; heterogeneity in the fine specificity of CD20 monoclonal antibodies is defined by additional requirements imposed by both amino acid sequence and quaternary structure. Blood 2002;99:3256–3262.

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

E-mail: yukinarikato@med.tohoku.ac.jp

Received: September 14, 2021
Accepted: January 11, 2022