MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 36, Number 2, 2017

DOI: 10.1089/mab.2017.0002

# Development of mPMab-1, a Mouse–Rat Chimeric Antibody Against Mouse Podoplanin

Shinji Yamada,[1,2] Mika K. Kaneko,[1,2] Takuro Nakamura,[1,2] Osamu Ichii,[3] Satoru Konnai,[3] and Yukinari Kato[1,2,4]

Podoplanin (PDPN), the ligand of C-type lectin-like receptor-2, is used as a lymphatic endothelial marker. We previously established clone PMab-1 of rat $IgG_{2a}$ as a specific monoclonal antibody (mAb) against mouse PDPN. PMab-1 is also very sensitive in immunohistochemical analysis; however, rat mAbs seem to be unfavorable for pathologists because anti-mouse IgG and anti-rabbit IgG are usually used as secondary antibodies in commercially available kits for immunohistochemical analysis. In this study, we develop a mouse–rat chimeric antibody, mPMab-1 of mouse $IgG_{2a}$, which was derived from rat PMab-1 mAb. Immunohistochemical analysis shows that mPMab-1 detects podocytes of the kidney, lymphatic endothelial cells of the colon, and type I alveolar cells of the lung. Importantly, mPMab-1 is more sensitive than PMab-1. This conversion strategy from rat mAb to mouse mAb could be applicable to other mAbs.

**Keywords:** podoplanin, monoclonal antibody, chimeric antibody, PMab-1

## Introduction

Podoplanin (PDPN) is expressed in many normal tissues such as podocytes of the kidney, lymphatic endothelial cells of many tissues, and type I alveolar cells of the lung.[1] Several anti-mouse PDPN monoclonal antibodies (mAbs) such as clone 8.1.1 or PMab-1 are commercially available.[1,2] However, 8.1.1 is produced using hamsters and PMab-1 is produced using rats because it is difficult to develop anti-mouse PDPN mAbs using mice. PMab-1 is produced against the platelet aggregation-stimulating domain of mouse PDPN[1]; therefore, PMab-1 neutralizes the interaction between PDPN and C-type lectin-like receptor 2 (CLEC-2). Administration of PMab-1 reduces lymphangiogenesis in the corneal suture and ear wound healing models.[3] PMab-1 also suppresses the infiltration of thioglycollate-induced macrophages at the site of wound healing. Furthermore, administration of PMab-1 leads to a significant suppression of the rejection reaction in the corneal transplantation model, suggesting that PDPN is a novel therapeutic target for suppressing lymphangiogenesis and inflammation.

In this study, we produce a mouse–rat chimeric antibody, mPMab-1, which is derived from PMab-1.

## Materials and Methods

### *Production of mPMab-1, a mouse–rat chimeric anti-mouse PDPN antibody*

PMab-1, a rat anti-mouse PDPN mAb, was developed as previously described.[1] To generate a mouse–rat chimeric anti-mouse PDPN (mPMab-1), appropriate $V_H$ cDNA of rat PMab-1 was subcloned into pFUSE-CHIGm$G_{2a}$ (InvivoGen, San Diego, CA) and appropriate $V_L$ cDNA of rat PMab-1 and $C_L$ of mouse kappa chain were subcloned into pCAG-Neo vector (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. Antibody expression vectors were transfected into CHO-S cells (Thermo Fisher Scientific, Inc., Waltham, MA) using the Lipofectamine LTX reagent (Thermo Fisher Scientific, Inc.). Stable transfectants of CHO-S/mPMab-1 were selected by cultivating the transfectants in a medium containing 0.5 mg/mL of both geneticin and zeocin (InvivoGen). CHO-S/mPMab-1 cells were cultivated in CHO-S-SFM II medium (Thermo Fisher Scientific, Inc.). mPMab-1 was purified using Protein G-Sepharose (GE healthcare Bio-Sciences, Pittsburgh, PA).

### *Flow cytometry*

CHO/mPDPN cells were established and cultured as described previously.[4] Cell lines were harvested by brief exposure to 0.25% Trypsin/1 mM EDTA (Nacalai Tesque, Inc., Kyoto, Japan). After washing, the cells were treated with PMab-1 or mPMab-1 (1 μg/mL) for 30 minutes at 4°C, followed by treatment with Oregon Green 488 goat anti-rat or anti-mouse IgG (Thermo Fisher Scientific, Inc.). Fluorescence data were collected using a Cell Analyzer EC800 (Sony Corp., Tokyo, Japan).

### *Immunohistochemical analyses*

This study used normal mouse tissues, which were isolated from one specific pathogen-free DBA/2Cr mouse (SLC

Departments of [1]Antibody Drug Development and [2]Regional Innovation, Tohoku University Graduate School of Medicine, Sendai, Japan.
[3]Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.
[4]New Industry Creation Hatchery Center, Tohoku University, Sendai, Japan.

Japan, Shizuoka, Japan). Four-micrometer-thick histologic sections were deparaffinized in xylene and rehydrated. After antigen retrieval using citrate buffer (pH 6.0; Dako, Glostrup, Denmark), sections were incubated with 1 μg/mL of PMab-1 or mPMab-1 for 1 hour at room temperature, followed by treatment with Envision+ kit (Dako) for 30 minutes. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (DAB; Dako) for 30 seconds, and then the sections were counterstained with hematoxylin (Wako Pure Chemical Industries Ltd.).

## Results and Discussion

We previously established PMab-1 of rat $IgG_{2a}$ as a specific mAb against mouse PDPN. PMab-1 neutralizes the interaction between PDPN and CLEC-2. PMab-1 is also very sensitive in the immunohistochemical analysis. In contrast, rat mAbs seem to be unfavorable for pathologists because anti-rat IgG is not included as secondary antibody in commercially available kits for immunohistochemical analysis such as Envision+ (Dako). Nevertheless, mouse anti-mouse PDPN antibodies have not been reported. Therefore, we herein produced a mouse–rat chimeric antibody, mPMab-1, which was derived from PMab-1.

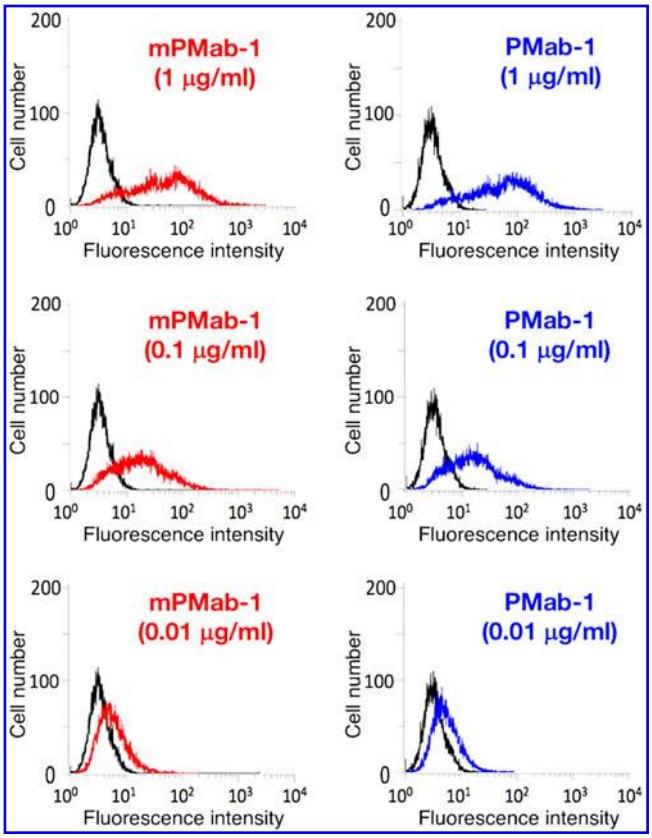


**FIG. 1.** Flow cytometric analysis of anti-mouse PDPN mAbs. Cells were treated with PMab-1 and mPMab-1 (1 μg/mL) or control PBS (black) for 30 minutes at 4°C, followed by treatment with anti-rat or mouse IgG Oregon Green. Fluorescence data were collected using a Cell Analyzer EC800. mAb, monoclonal antibody; PBS, phosphate-buffered saline; PDPN, podoplanin.

We first checked the reactivity of mPMab-1 in flow cytometry. The reactivity of mPMab-1 against CHO/mPDPN cells is compatible to that of PMab-1 (Fig. 1). To investigate whether mPMab-1 is useful in immunohistochemical analysis using commercially available kits, we next compared the reactivity between PMab-1 and mPMab-1 against normal mouse tissues. mPMab-1 reacted with renal podocytes (Fig. 2A, B) in the same way as PMab-1 (Fig. 2C, D). Importantly, the reactivity of mPMab-1 is higher than that of PMab-1. Without primary antibodies, slight unspecific staining was observed in red blood cells of the renal glomerulus (Supplementary Fig. S1A, B). Both mPMab-1 (Fig. 3A, B) and PMab-1 (Fig. 3C, D) also stained lymphatic endothelial cells and fibroblasts of the colon. The signal of mPMab-1 is also higher compared with PMab-1. Furthermore, both mPMab-1 (Fig. 4A, B) and PMab-1 (Fig. 4C, D) stained type I alveolar cells of the lung, indicating that mPMab-1 is very useful for immunohistochemical analysis using commercially available immunohistochemical kits.

In this study, PMab-1 was also shown to be useful in immunohistochemical analysis, although anti-mouse secondary antibodies are included in immunohistochemical kits. Anti-mouse secondary antibodies usually cross-react with rat antibodies; cross-reaction seems to possess a lower reliability for pathologists and scientists. mPMab-1 could be used as mouse IgG and its reactivity is higher than that of PMab-1. This conversion strategy from rat mAb to mouse antibody could be applicable to other mAbs.

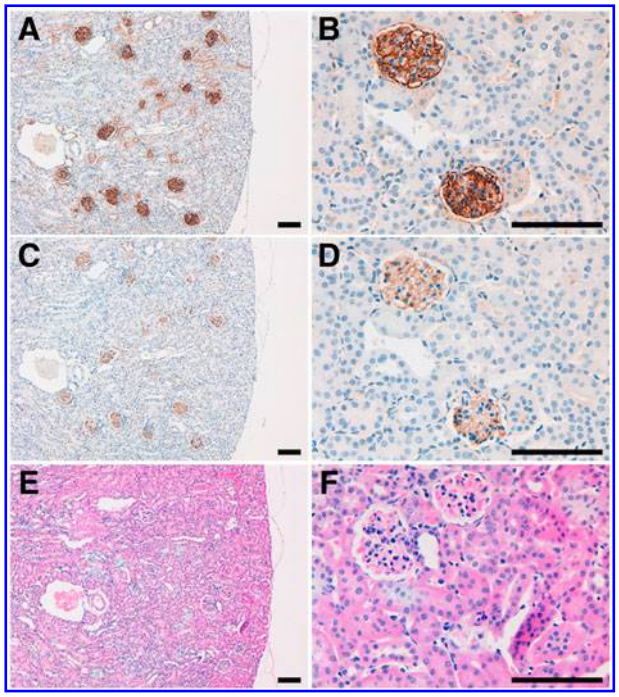


**FIG. 2.** An immunohistochemical analysis using anti-mouse PDPN mAbs to detect PDPN expression in the kidney. Sections were incubated with mPMab-1 **(A, B)** or PMab-1 **(C, D)** and made to react with the Envision+ kit. Color was developed using DAB, and samples were then counterstained with hematoxylin. Sections were also stained with hematoxylin and eosin **(E, F)**. Scale bar = 100 μm.

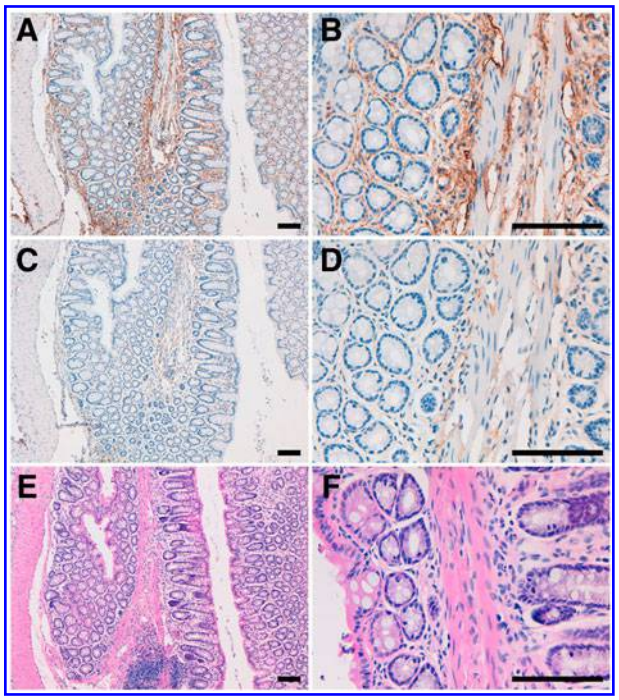


**FIG. 3.** An immunohistochemical analysis using anti-mouse PDPN mAbs to detect PDPN expression in the colon. Sections were incubated with mPMab-1 **(A, B)** or PMab-1 **(C, D)** and made to react with the Envision+ kit. Color was developed using DAB, and samples were then counter-stained with hematoxylin. Sections were also stained with hematoxylin and eosin **(E, F)**. Scale bar = 100 μm.

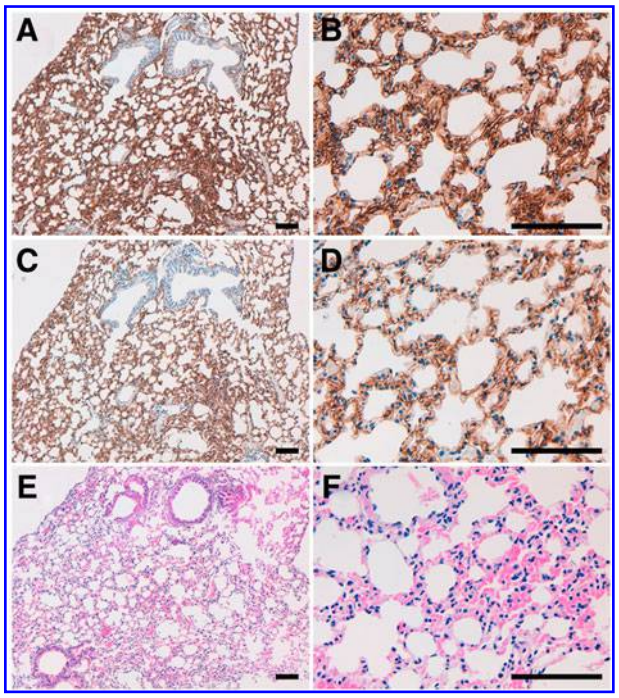


**FIG. 4.** An immunohistochemical analysis using anti-mouse PDPN mAbs to detect PDPN expression in the lung. Sections were incubated with mPMab-1 **(A, B)** or PMab-1 **(C, D)** and made to react with the Envision+ kit. Color was developed using DAB, and samples were then counter-stained with hematoxylin. Sections were also stained with hematoxylin and eosin **(E, F)**. Scale bar = 100 μm.

## Acknowledgments

The authors thank Miyuki Yanaka, Noriko Saidoh, and Kanae Yoshida for their excellent technical assistance. They also thank Satoshi Ogasawara, Yuki Fujii, Hiroaki Uchida, and Hideaki Tahara for their specialized advice. This work was supported, in part, by the Basic Science and Platform Technology Program for Innovative Biological Medicine from Japan Agency for Medical Research and development, AMED (Y.K.), by the Translational Research Network Program from AMED (Y.K.), by a project for utilizing glycans in the development of innovative drug discovery technologies from AMED (Y.K.), by the Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS) from AMED (Y.K.), by JSPS KAKENHI Grant Number 26440019 (M.K.K.) and 16K10748 (Y.K.), and by the Regional Innovation Strategy Support Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (Y.K.). This work was performed, in part, under the Cooperative Research Program of Institute for Protein Research, Osaka University, CR-16-05, and by the Grant for Joint Research Project of the Institute of Medical Science, the University of Tokyo. The authors would like to thank Enago (www.enago.jp) for the English-language review.

## Author Disclosure Statement

No competing financial interests exist.

## References

1. Kaji C, Tsujimoto Y, Kato Kaneko M, Kato Y, Sawa Y: Immunohistochemical examination of novel rat monoclonal antibodies against mouse and human podoplanin. Acta Histochem Cytochem 2012;45:227–237.
2. Schacht V, Dadras SS, Johnson LA, Jackson DG, Hong YK, Detmar M: Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Pathol 2005;166:913–921.
3. Maruyama Y, Maruyama K, Kato Y, Kajiya K, Moritoh S, Yamamoto K, Matsumoto Y, Sawane M, Kerjaschki D, Nakazawa T, Kinoshita S: The effect of podoplanin inhibition on lymphangiogenesis under pathological conditions. Invest Ophthalmol Vis Sci 2014;55:4813–4822.
4. Kato Y, Fujita N, Kunita A, Sato S, Kaneko M, Osawa M, Tsuruo T: Molecular identification of Aggrus/T1alpha as a platelet aggregation-inducing factor expressed in colorectal tumors. J Biol Chem 2003;278:51599–51605.

Address correspondence to:
*Dr. Yukinari Kato*
*New Industry Creation Hatchery Center*
*Tohoku University*
*2-1 Seiryo-machi*
*Aoba-ku*
*Sendai 980-8575*
*Japan*

*E-mail:* yukinari-k@bea.hi-ho.ne.jp

*Received: January 17, 2017*
*Accepted: February 28, 2017*