



Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Original Article

Characterization of human decidual mast cells and establishment of a culture system

Takayuki Matsuno^{a, b, 1}, Shota Toyoshima^{b, c, 1}, Tomomi Sakamoto-Sasaki^{b, c},
Jun-ichi Kashiwakura^d, Akira Matsuda^e, Yasuo Watanabe^e, Hiromitsu Azuma^a,
Kei Kawana^a, Tatsuo Yamamoto^f, Yoshimichi Okayama^{b, c, *}

^a Department of Obstetrics and Gynecology, Nihon University School of Medicine, Tokyo, Japan^b Allergy and Immunology Project Team, Nihon University School of Medicine, Tokyo, Japan^c Center for Institutional Research and Medical Education, Nihon University School of Medicine, Tokyo, Japan^d Department of Immunology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan^e Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan^f Department of Obstetrics and Gynecology, Kasukabe Medical Center, Saitama, Japan

ARTICLE INFO

Article history:

Received 29 January 2018

Received in revised form

12 April 2018

Accepted 25 April 2018

Available online xxx

Keywords:

Cultured decidual-derived mast cells

Decidua

FcεRI

Mast cell

Pregnancy

Abbreviations:

Ab, antibody; CB, cord blood; EIA, enzyme immunoassay; EM, electron microscopic analyses; IL, interleukin; IMDM, Iscove's modified Dulbecco's medium; m, monoclonal; MCs, mast cells; MC_T, MCs whose granules contain tryptase only; MC_{TC}, MCs whose granules contain tryptase, chymase, carboxypeptidase and a cathepsin G-like enzyme; MFI, mean fluorescence intensity; NK, natural killer; rh, recombinant human; SCF, stem cell factor; Treg, regulatory T

ABSTRACT

Background: Although rodent decidual mast cells (MCs) reportedly play an important role in implantation and placenta formation, the characterization of human decidual MCs has been not well clarified. The aims of this study were to investigate the distribution and characteristics of MCs in human decidua and to establish a culture system for decidua-derived MCs.

Methods: Decidual tissues were obtained from patients who underwent a legal elective abortion (6th week to 9th week of pregnancy), and decidual MCs were enzymatically dispersed. Cultured decidual-derived MCs were generated by culturing decidual cells with stem cell factor. An ultrastructural analysis of primary decidual MCs and cultured decidual-derived MCs was performed using a transmission electron microscope. Receptor and protease expression was analyzed using FACS. Histamine released from MCs was measured using enzyme immune assays.

Results: A larger proportion of tryptase positive⁽⁺⁾ MCs in decidua was present on the maternal side. Both enzymatically dispersed decidual MCs and cultured decidual-derived MCs showed an FcεRIα⁺Kit⁺ tryptase⁺chymase⁺ phenotype. Their granules containing particles exhibited variable amounts of electron-lucent space separating electron-dense particles. Both enzymatically dispersed decidual MCs and cultured decidual-derived MCs released comparable amounts of histamine following FcεRI aggregation.

Conclusions: The isolation method for MCs from decidua during early pregnancy and the culture system for decidual-derived MCs may enable the roles of decidual MC during pregnancy to be explored.

Copyright © 2018, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The fetus is a semi-allograft that carries paternal antigens for the mother. Despite this, fetuses are not rejected, enabling pregnancies to be maintained. One part of the human placenta, i.e. the decidua basalis, is particularly important for local immunity during early pregnancy. The decidua basalis consists of transformed endometrium located at the site of embryo implantation.^{1,2} Pregnancy sustenance mechanisms including immune tolerance are

* Corresponding author. Allergy and Immunology Project Team, Center for Institutional Research and Medical Education, Nihon University School of Medicine, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo, 173-8610, Japan.

E-mail address: okayama.yoshimichi@nihon-u.ac.jp (Y. Okayama).

Peer review under responsibility of Japanese Society of Allergy.

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.alit.2018.05.001>

1323-8930/Copyright © 2018, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

considered to be established in the decidua. Natural killer (NK) cells, macrophages, regulatory T (Treg) cells, regulatory B cells and T cells in the decidua are involved in the pregnancy sustenance mechanisms.^{1,2} Mast cells (MCs) also exist in the decidua.³ However, the characteristics of human decidual MCs and the role of MCs in pregnancy have not been well clarified.

MCs play a central role in IgE-dependent allergic diseases such as asthma and rhinoconjunctivitis through the release of a variety of vasoactive and bronchospastic autacoid mediators and functionally diverse proteases, chemokines and cytokines.⁴ MCs also reportedly exist in the human uterus.⁵ Studies on rodents have suggested that MCs in the uterus are involved in implantation,⁶ placenta formation,⁷ and uterine contraction.⁸ MC depletion reportedly results in abnormally remodeled spiral arteries and intrauterine growth restriction.^{6,7} Furthermore, mice deficient in both NK cells and MCs show markedly impaired spiral artery remodeling and fetal growth-retardation.^{7,9} Meyer et al. found that α -chymase mast cell protease 5 mediates the apoptosis of uterine smooth muscle cells, a key feature of spiral arteries remodeling.⁷ Chymase secreted by uterine MCs and uterine NKs are pivotal to the vascular changes required to support pregnancy. The only human chymase is α -chymase,¹⁰ the phylogenetic homolog of mouse α -chymase mast cell protease 5.¹¹ Therefore, investigating the localization of MCs in decidua and whether decidual MCs are MC_T type (MCs whose granules contain tryptase only) or MC_{TC} type (MCs whose granules contain tryptase, chymase, carboxypeptidase and a cathepsin G-like enzyme) is important. Some reports have discussed uterine MCs and their relationship with premature birth, contraction, and delivery.⁸ However, no contraction abnormalities or abnormal delivery periods were seen in mice that lack MCs.¹² These findings seem to suggest that uterine MCs are not a prerequisite for contraction and delivery. The role of human decidual MCs in implantation and placenta formation is not yet fully understood. To address this issue, we investigated the localization of decidual MCs, MC phenotypes and their functions and established a culture system that is fundamental to understanding the characteristics of decidual MCs.

Methods

Ethical considerations

This study was approved by the Ethics Committee of the Nihon University School of Medicine, and all the subjects provided written informed consent in accordance with the Helsinki Declaration of the World Medical Association. The approval number from the Ethical Committee was RK-150609-16.

Reagents

The following antibodies (Abs) were purchased from the indicated sources: APC-conjugated anti-Fc ϵ R1 α monoclonal Ab (mAb, clone CRA1; Biolegend, San Diego, CA, USA), PE-conjugated anti-human Kit mAb (clone YB5. B8, BD Bioscience, San Diego, CA, USA), anti-human tryptase mAb (clone AA1; GeneTex, Irvine, CA, USA), and biotinylated anti-human chymase mAb (clone B7; Chemicon International, Temecula, CA, USA).

Immunohistochemical analysis

Fresh samples of decidual tissues were obtained from women who underwent a legal elective abortion (6th week to 9th week of pregnancy) at Narimasu Maternity & Women's Care Hospital, Tokyo, Japan, after obtaining informed consent. Each specimen was immediately placed in OCT medium, snap-frozen in liquid

nitrogen, and stored at -80°C until cryostat sectioning. The cells were stained with Alexa Fluor[®] 488-labeled anti-tryptase mAb (clone AA1).

Electron microscopic analyses (EM)

EM was performed as described previously.¹³ Briefly, decidual tissues or cultured decidual-derived MCs were fixed with 2.5% glutaraldehyde and further fixed with 2% osmic acid. The tissues were then embedded in epoxy resin and ultrathin sections (60–80 nm) were prepared. For EM using fixed MCs, cytospinning was performed. These samples were then examined using a transmission electron microscope (7000-100; Hitachi High-Technologies, Tokyo, Japan).

Purification and generation of cultured decidual-derived MCs

Human cultured decidual-derived MCs were generated using a previously described method for the generation of cultured lung-derived MCs with some modifications.¹⁴ Briefly, fresh samples of decidual tissues were obtained from 30 women who underwent a legal elective abortion at Narimasu Maternity & Women's Care Hospital, Tokyo, Japan, after obtaining informed consent. The decidual cells were enzymatically dispersed using 1.5 mg/mL of collagenase type I (Sigma–Aldrich, St Louis, MO, USA), 0.75 mg/mL of hyaluronidase (Sigma–Aldrich) and 0.1% BSA in Iscove's Modified Dulbecco's Medium (IMDM; Invitrogen, Grand Island, NY, USA) for 30 min at 37°C . For enrichment of the MCs, the dispersed cells were suspended in 30% Percoll (GE Healthcare, Little Chalfont, UK) and the cell suspension was placed on 70% Percoll. The cells were centrifuged, and the decidual MC progenitor cells and mature decidual MCs at the 70% Percoll interface were collected and washed. The cells were then cultured in serum-free Iscove's methylcellulose medium (Stem Cell Technologies, Vancouver, BC, Canada) and IMDM supplemented with 100 ng/mL of recombinant human stem cell factor (rhSCF) (PeproTech, Rocky Hill, NJ, USA) and 50 ng/mL of rh interleukin (IL)-6 (PeproTech). On day 42, methylcellulose was dissolved in PBS, and the cells were resuspended and cultured in IMDM containing 0.1% BSA, 100 ng/mL of rhSCF, and 50 ng/mL of rhIL-6 (designated as MC medium). The purity of human decidual MCs, as assessed using metachromatic staining, was more than 80%.

Purification and generation of cultured human synovial MCs

Fresh samples of synovial tissues from eight donors undergoing a total knee arthroplasty at Nihon University were obtained after the donors had provided informed consent. Synovial cells were enzymatically dispersed as described previously (purity of MCs was ~5%).¹³ For MC enrichment, the dispersed cells were resuspended in IMDM supplemented with 0.5% BSA and 100 U/mL of penicillin-streptomycin, then centrifuged using a density gradient consisting of 22.5% HistoDenz solution (Sigma–Aldrich) and lymphocyte separation medium (LSM; Organon Teknica, Durham, NC). The synovial MC progenitor cells and mature synovial MCs were collected from the cell pellets at the bottom of the centrifuged tube and the cell band at the LSM interface. The purity of metachromatic staining-positive⁽⁺⁾ cells was $43\% \pm 4\%$. The collected MC progenitor cells and mature MCs were incubated in IMDM containing 0.1% BSA, 100 ng/mL of rhSCF (PeproTech EC), and 50 ng/mL of rhIL-6 (PeproTech EC) for 24–48 h.

Generation of cultured cord blood (CB)-derived MCs

Human CB CD34⁺ cells were supplied from RIKEN BioResource Center, Tsukuba, Ibaraki, Japan. Human CB CD34⁺ cells were

cultured in serum-free Iscove methylcellulose medium (Stem Cell Technologies) and IMDM containing rhSCF (PeproTech EC) at 200 ng/mL, rhIL-6 (PeproTech EC) at 50 ng/mL, and rhIL-3 (Intergen Purchase, NY, USA) at 1 ng/mL, as previously described.¹⁵ On day 42, methylcellulose was dissolved in PBS and the cells were resuspended and cultured in IMDM containing 0.1% BSA, 100 ng/mL of rhSCF, and 50 ng/mL of rhIL-6. The purity of human MCs, as assessed using metachromatic staining, was more than 98%.

Flow cytometric analysis

The expressions of FcεRIα and Kit on the surfaces of cells were analyzed using flow cytometric analysis. Briefly, MCs were collected and fixed with 2% paraformaldehyde for 20 min at 4 °C. Then, the MCs were treated with Human TruStain FcX™ (Biolegend, San Diego, CA, USA). The cells were washed and resuspended in 0.1% BSA-PBS. APC-conjugated anti-FcεRIα mAb (clone CRA1) and PE-conjugated anti-human Kit mAb (clone Yb5B8) were added to the cell suspension, and the cells were incubated for 20 min at 4 °C. In some experiments, intracellular staining of tryptase and chymase was performed using the BD Cytofix/Cytoperm™ Kit (BD Biosciences), followed by staining with Alexa Fluor® 488-conjugated anti-human tryptase mAb (clone AA1) and biotinylated anti-human chymase mAb (clone B7). The cells stained with biotinylated anti-human chymase mAb were incubated with PE/Cy7-conjugated streptavidin (BD Biosciences) for 30 min at 4 °C. The expression on MCs was detected using FACS Gallios (Beckman Coulter, Brea, CA, USA) or FACS Calibur (BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo (Tree Star, Ashland, OR, USA).

MC activation

For the aggregation of FcεRI, MCs were sensitized with 0.5 μg/mL of human IgE (Calbiochem, San Diego, CA, USA) for 30 min at 37 °C. The cells were washed once and resuspended in HEPES buffer or MC medium. The IgE-sensitized MCs were stimulated with anti-human IgE (DakoCytomation, Carpinteria, CA, USA) for 30 min for the histamine enzyme immunoassay (EIA, 2×10^3 MCs/100 μL). The MCs were challenged by incubation with 10^{-6} M calcium ionophore A23187 (Sigma–Aldrich) at 37 °C for 30 min for the measurement of histamine release. After incubation, cell-free culture supernatants and cell pellets were harvested to measure the mediator levels.

Histamine assay

Histamine was measured using an EIA kit (Beckman Coulter, Indianapolis, IN, USA). The percent histamine release was calculated as follows: (histamine release/total histamine content of unstimulated MCs) × 100%.

Statistical analysis

The differences between independent *in vitro* experiments were analyzed using the Mann–Whitney U test. The one way ANOVA and Dunnett's tests were used for comparing the differences between more than three groups. The *P* value was considered significant at *P* < 0.05.

Results

To investigate the localization of MCs in decidua, we performed an immunohistochemical analysis of the decidua using anti-human tryptase mAb. Tryptase⁺ cells were mainly localized on

the maternal side (Fig. 1A). The number of decidual tryptase⁺ cells was 2.1 ± 0.5 cells per high power field (the mean ± SEM, *n* = 4 donors, Fig. 1C). As negative controls, decidua was stained with mouse IgG1 (Fig. 1B).

Next, we enzymatically dispersed decidual cells from the decidual tissues of abortion specimens obtained during early pregnancy (mean decidual tissue weight, 4.9 ± 2.7 g) and enriched the MCs using Percoll density gradient centrifugation. The total cell count isolated per 1 g of decidual tissues was $8.2 \times 10^6 \pm 9.3 \times 10^6$ (*n* = 12 donors), and $14.8 \times 10^5 \pm 18.3 \times 10^5$ cells per 1 g of decidual

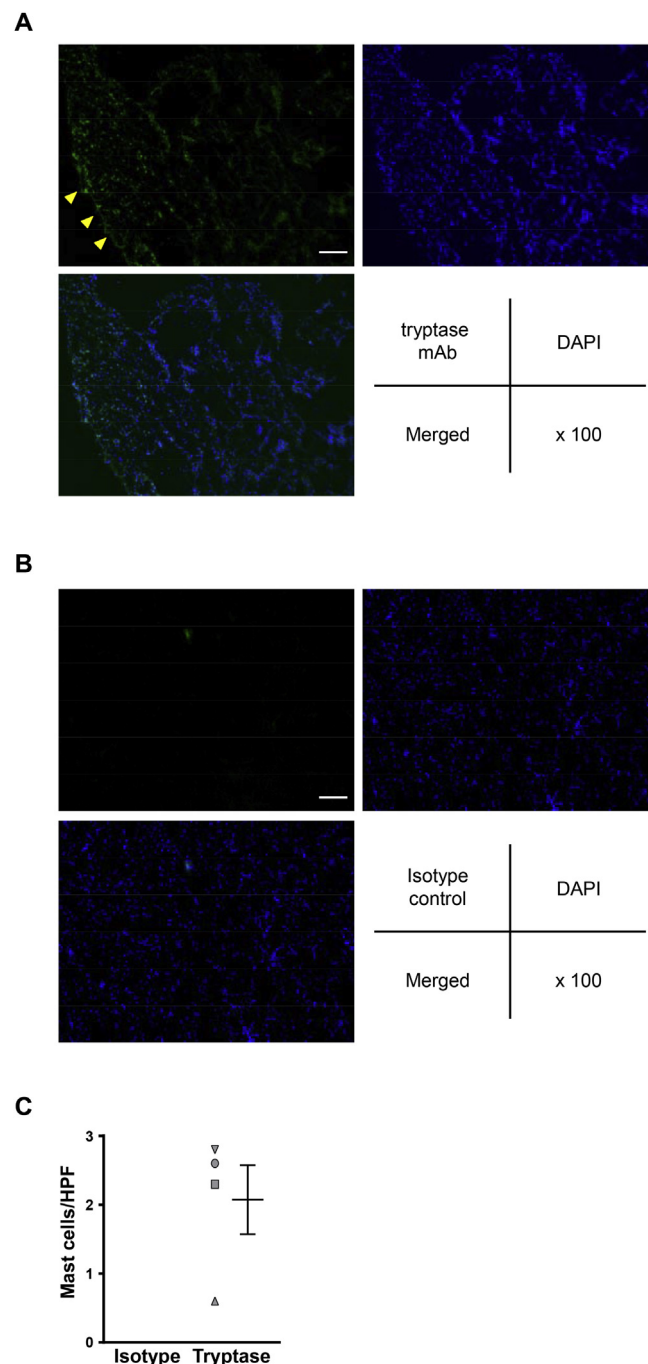


Fig. 1. Localization of MCs in decidua. Representative images of MCs (tryptase⁺ cells) in decidual tissues (A). Immunohistochemical staining of decidua with anti-tryptase (green), DAPI (blue) and a merged image (yellow). (B) As negative controls, decidua was stained with mouse IgG1. Bar = 20 μm. (C) Number of MCs per high power field (HPF, scatter plot, *n* = 4 donors). The means and SEM are also shown.

tissues were metachromatic staining⁺ cells. The purity of the decidual metachromatic staining⁺ cells after Percoll density gradient centrifugation was $16.9 \pm 8.0\%$.

We examined the morphology of MCs in decidia using electron microscopy. The decidual MCs have a single lobed nucleus and various granules in the cytoplasm. Their granules containing particles exhibited variable amounts of electron-lucent space separating electron-dense particles (Fig. 2A). To analyze the phenotype of decidual Kit⁺FcεRIα⁺ cells, a gate was set up for decidual Kit⁺FcεRIα⁺ cells to examine the expressions of tryptase and chymase (Fig. 3A and B, left panels). Although both expressions were observed, the mean fluorescence intensity (MFI) ratio of the tryptase expression was 47.0 ± 22.3 (Fig. 3A, n = 5 donors, right panel), while the MFI ratio of the chymase expression was 6.6 ± 1.4 (Fig. 3B, n = 5 donors, right panel).

We tried to culture semi-purified MCs (~17% purity) in a methylcellulose culture medium (see Methods) and observed MC colonies (data not shown). We determined the number and percentage of MCs in the culture population using metachromatic staining. Following gradient centrifugation of the dispersed decidual cells, the purity of the metachromatic staining⁺ cells was 17% (day 0, n = 12 donors). After 11 weeks, the percentage of metachromatic staining⁺ cells increased to more than 80% (Fig. 2C). The number of metachromatic staining⁺ cells gradually increased during the culture and reached 1.0×10^5 at 11 weeks. We examined the morphologies of the 11-week-cultured decidia-derived MCs using light and electron microscopy. The cultured decidia-derived MCs were toluidine blue-positive cells (Fig. 2D). As can be seen in Fig. 2B, the cultured decidia-derived MCs had granules containing particles that exhibited variable amounts of electron-lucent space separating electron-dense particles, indicating that the granules appeared to be similar to those of MCs in decidual tissues (Fig. 2A). As can be seen in Fig. 2C, the purity of 8-week-cultured decidia-

derived MCs varied, with the purity of cultured decidia-derived MCs peaking at 11 weeks. Thus, we compared the expressions of tryptase and chymase among 11-week-cultured decidia-derived MCs, cultured synovium-derived MCs and cultured CB-derived MCs using flow cytometry (Fig. 3C–H). Using intracellular staining, the averages of MFI ratio of the tryptase and chymase expressions of cultured decidia-derived MCs were 3.7 and 3.2, respectively (Fig. 3C and D). The tryptase expression levels of the three cultured MCs were comparable, whereas the chymase expression level of the cultured decidia-derived MCs was lowest. When the tryptase and chymase expression levels of the cultured decidia-derived MCs were compared with those of the primary decidual MCs, the chymase expression level was similar to that of the primary decidual MCs, whereas the expression level of tryptase was lower than that of the primary decidual MCs.

To compare the functions between the primary decidual MCs and the cultured decidia-derived MCs, histamine release from the MCs triggered by IgE-dependent stimulation was examined. Under our experimental conditions, both MCs released comparable amounts of histamine through IgE-dependent stimulation (Fig. 4A and B). We compared the histamine contents in the primary decidual MCs, cultured decidia-derived MCs, cultured CB-derived MCs and cultured synovium-derived MCs (Fig. 4C) and obtained values of 31.8 ± 12.7 nmol/ 10^6 cells (n = 3 donors), 25.5 ± 9.9 nmol/ 10^6 cells (n = 5 donors), 4.2 ± 1.0 nmol/ 10^6 cells (n = 3 donors) and 17.8 ± 2.7 nmol/ 10^6 cells (n = 4 donors), respectively.

Discussion

The present study successfully established a culture system of decidia-derived MCs obtained during early pregnancy (Fig. 2B–D). An examination of the protease expression pattern of human primary decidual MCs obtained during early pregnancy and cultured

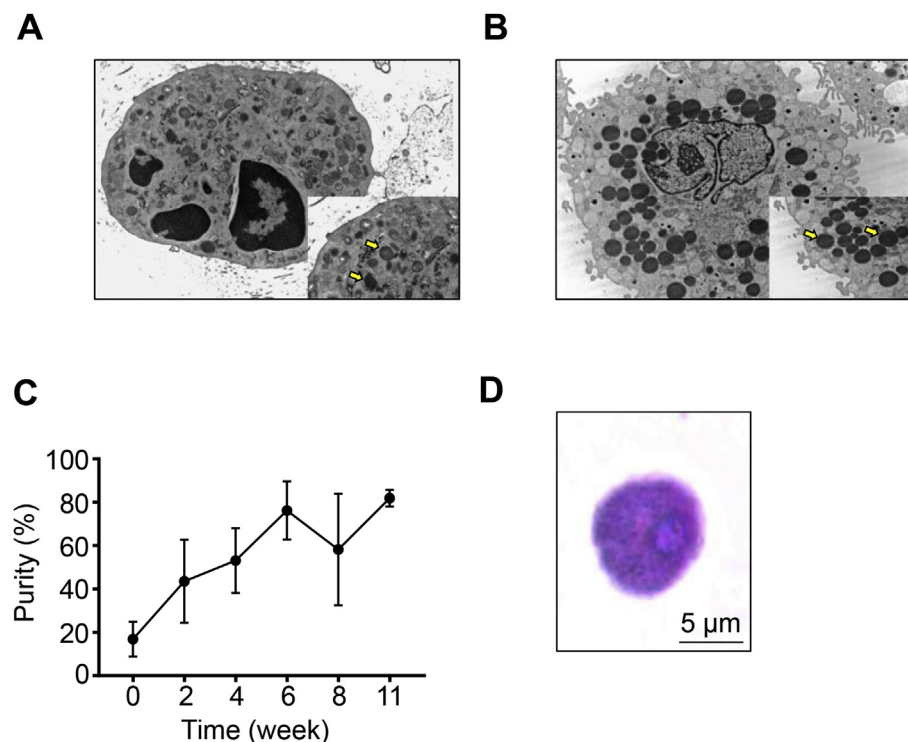


Fig. 2. Generation of cultured decidia-derived MCs. (A) and (B) Granule-type analysis of primary decidual MCs (A) and cultured decidia-derived MCs using EM. Insets of A and B, Magnified images of granules in (A) and (B). The yellow arrows indicate granules. (C) Purity of cultured decidia-derived MCs at the indicated culture time points. Data are the mean $\pm$ SD (n = 2 independent experiments). (D) Metachromatic staining of cultured decidia-derived MCs. Bar = 5 μ m.

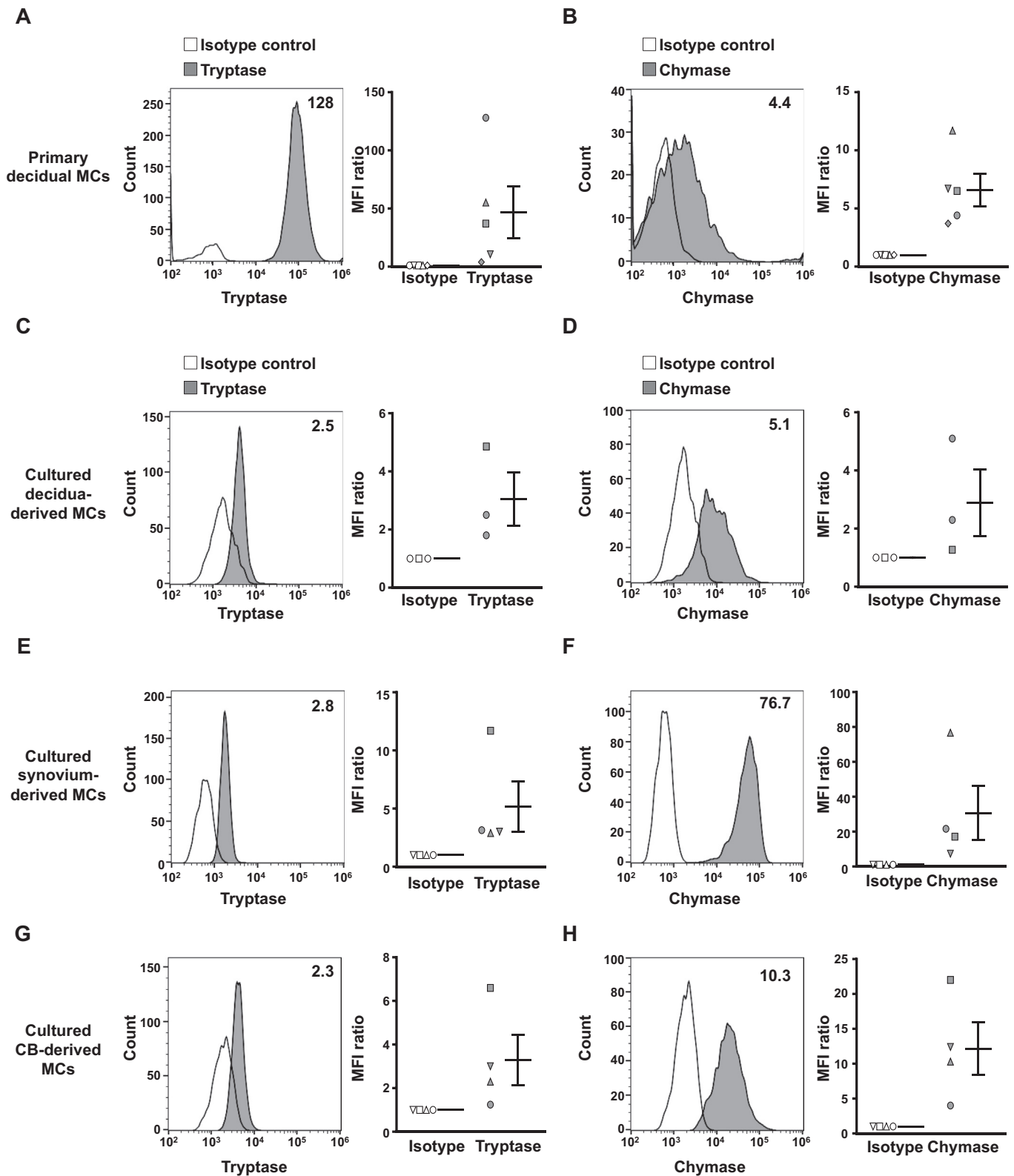


Fig. 3. Analysis of tryptase and chymase on FcεRIα⁺ Kit⁺-gated decidual cells using FACS. (A) and (B) Representative flow cytometric images of tryptase and chymase in FcεRIα⁺ Kit⁺-gated primary decidual cells, respectively (left panels). The averages of the MFI ratio of tryptase and chymase expression in primary decidual MCs are shown (right panels, mean ± SEM of 5 independent experiments). Data are also expressed as the mean ± SEM. (C), (D) and (E) Representative flow cytometric images of tryptase and chymase expression in cultured decidual-derived MCs (C and D, left panels), cultured synovium-derived MCs (E and F, left panels) and cultured CB-derived MCs (G and H, left panels). The averages of the MFI ratio of tryptase and chymase expression in these cultured MCs are shown (right panels, mean ± SEM of 3–4 independent experiments). Data are also expressed as the mean ± SEM. The numbers in the histograms indicate the MFI ratio of the expression levels of tryptase and chymase to those of the isotype control (mouse IgG1). The positive and negative gates for tryptase and chymase staining are set using an isotype control. Different symbols indicate individual donors.

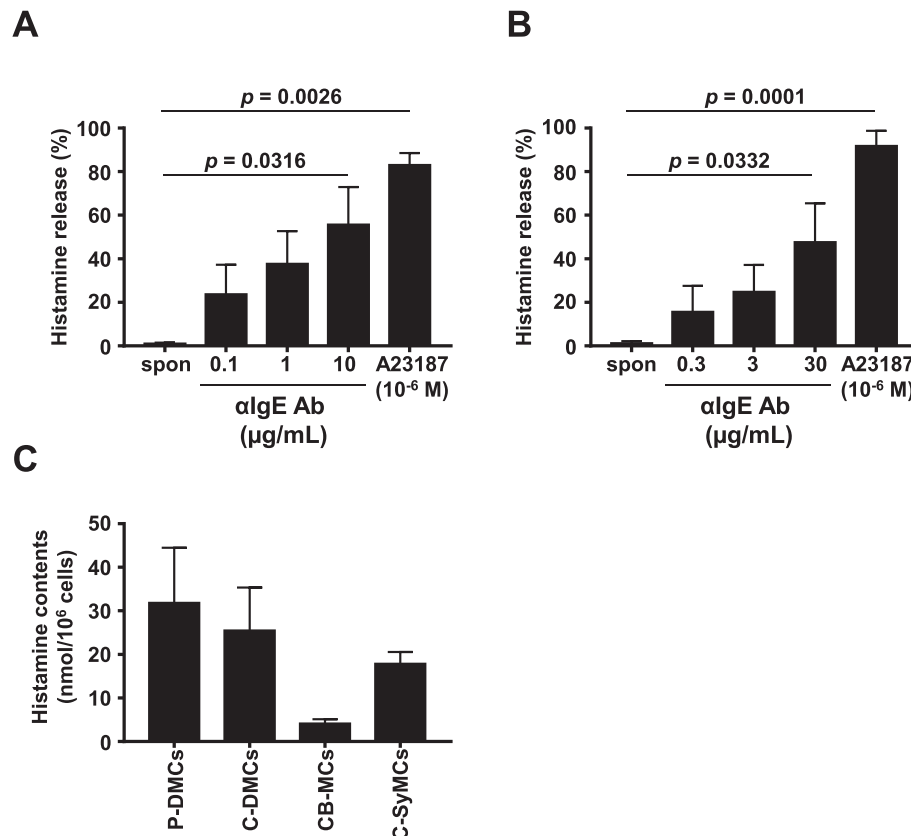


Fig. 4. Histamine release from primary decidual MCs (A, $n = 3$) and cultured decidual-derived MCs (B, $n = 5$) following the aggregation of Fc ϵ RI. MCs were stimulated with anti-IgE (α IgE) Ab or calcium ionophore A23187. Spontaneous histamine release from primary decidual MCs and cultured decidual-derived MCs was observed for $1.0\% \pm 0.6\%$ and $1.2\% \pm 1.0\%$, respectively. (C) Comparison of the histamine contents of the primary decidual MCs (P-DMCs), cultured decidual-derived MCs (C-DMCs), cultured CB-derived MCs (CB-MCs) and cultured synovium-derived MCs (C-SyMCs). Data are the mean $\pm$ SEM. spon; spontaneous release.

decidua-derived MCs using flow cytometry revealed that the pattern was tryptase⁺chymase⁺ MC_{TC} type (Fig. 3). Furthermore, the decidual MCs were confirmed to have histamine productivity in both cultured decidua-derived MCs and the primary decidual MCs (Fig. 4). Granules containing particles similarly exhibited variable amounts of electron-lucent space separating electron-dense particles (Fig. 2A and B, insets). The isolation method for MCs obtained from decidua during early pregnancy and the culture system for decidua-derived MCs are expected to enable further exploration of the roles of decidual MC in pregnancy.

In MCs from human skin and lung, the expression of G protein-coupled receptor was maintained in long-term cultures^{14,16} and a protease phenotype has also been maintained in long-term cultures. However, the expression level of tryptase in the cultured decidua-derived MCs was lower than that in the primary decidual MCs. Thus, the cultured decidua-derived MCs were not the same as the MCs found locally in the decidua. We previously isolated MCs from the human joint synovium and compared the granule structure of the MCs immediately after isolation with that of cultured synovium-derived MCs using an electron microscope, reporting that they had the same granule structure and that the characteristics of the granular proteases were kept intact (MC_{TC}).¹³ Furthermore, we compared receptors that were being expressed on the cell surface, and reported that no difference was found in the expression levels of Kit, Fc γ RI, Fc γ RIIA, Fc γ RIII, and Fc ϵ RI, while a difference in the expression levels of Fc γ RIIB was seen.¹³ Therefore, we are currently planning to compare the differences with cultured MCs by sorting MCs from the human decidua using a DNA chip.

Since the purity of primary decidual MCs and that of the cultured decidua-derived MCs differed, contaminant cells in

enriched MC populations might affect the release of histamine from MCs. However, we confirmed that the contamination of basophils was negligible in either of the enriched MC populations (data not shown). Under the experimental conditions, both MCs released comparable amounts of histamine through IgE-dependent stimulation (Fig. 4A and B). The histamine content of primary decidual MCs was comparable to that of cultured decidua-derived MCs (Fig. 4C). The histamine content of primary decidual MCs (31.8 ± 12.7 nmol/ 10^6 cells) or cultured decidua-derived MCs (25.5 ± 9.9 nmol/ 10^6 cells) was much higher than that of cultured synovium-derived MCs (17.8 ± 2.7 nmol/ 10^6 cells), primary lung MCs (17.0 ± 2.1 nmol/ 10^6 cells),¹⁷ primary children's foreskin MCs (4.3 ± 1.0 nmol/ 10^6 cells),¹⁷ and cultured CB-derived MCs (4.2 ± 1.0 nmol/ 10^6 cells). These results provide new evidence of human MC heterogeneity. Since in human, histamine is reportedly involved in blastocyst implantation, the invasion and proliferation of villi, and the development of an adhesive molecule leading to placenta formation,⁸ histamine release from decidual MCs might associate with placenta formation.

Treg is an important cell for pregnancy sustenance. Woidacki et al.¹⁸ reported that uterine MCs decreased with abortion-prone mice (DBA/2-mated CBA/J females), and the adoptive transfer of Treg into these mice increased uterine MCs and improved the remodeling of spiral arteries, thus reducing the miscarriage rate; these findings suggest that MCs play key roles in immune tolerance. Furthermore, the data suggests an interplay between Tregs and uterine MCs that is relevant for the changes required at the fetomaternal interface during the normal progression of pregnancy. One such mechanism is cell-to-cell interactions with Treg via OX40L-OX40.¹⁹ MCs play an important role in Treg-mediated

allograft tolerance, and Treg activates MCs or aggregates MCs via IL-9.^{20,21} Future exploration of the relationship between decidual MCs and Treg should unveil the role of MCs in immune tolerance during pregnancy. Furthermore, MCs accumulate on the maternal side in the decidua, suggesting that MCs play an important role in pregnancy sustenance mechanisms. We demonstrated that decidual MCs contain chymase. Actually, chymase-producing cells of the innate immune system are reportedly required for decidual vascular remodeling and fetal growth, while human chymase induces the migration of extravillous trophoblasts.⁷ Thus, human MC chymase might induce decidual vascular remodeling and fetal growth on the maternal side of the decidua.

In the future, this approach will enable us to explore the mechanisms of implantation and placenta formation. Furthermore, we hope that our findings will contribute to the exploration of disease mechanisms using flow cytometry and DNA chips to compare and analyze decidual MCs in patients who exhibit pregnancy complications, such as recurrent miscarriage, premature birth, pregnancy-induced hypertension, and intrauterine growth restriction with decidual MCs in patients with normal pregnancies.

Acknowledgements

We thank the staff members of Narimasu Maternity & Women's Care Hospital, Tokyo, Japan and the staff members of Department of Orthopedic Surgery, Nihon University School of Medicine, Tokyo, Japan for supplying the human specimens and for their willing and capable assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government (Project No. (C) 15K09558, awarded to Y.O.), the Nihon University Multidisciplinary Research Grant for 2015–2016 (Project No. So15-010 and So-16-014, awarded to Y.O.), and the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2015–2019 (Project No. S1511014, awarded to Y.O.).

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

TM, ST, TSS, JK, AM and YW performed research and analyzed the data. YO designed the study, analyzed the data and wrote the manuscript. HA, KK and TY supervised TM's work. HA and TY contributed to the collection of human samples.

References

1. Le Bouteiller P, Bensussan A. Up-and-down immunity of pregnancy in humans. *F1000Res* 2017;**6**:1216.

2. Ghaebi M, Nouri M, Ghasemzadeh A, Farzadi L, Jadidi-Niaragh F, Ahmadi M, et al. Immune regulatory network in successful pregnancy and reproductive failures. *Biomed Pharmacother* 2017;**88**:61–73.
3. Needham K, Fadia M, Dahlstrom JE, Harrington K, Shadbolt B, Robson SJ. Significance of mast cell distribution in placental tissue and membranes in spontaneous preterm birth. *J Inflamm Res* 2016;**9**:141–5.
4. Okayama Y, Kawakami T. Development, migration, and survival of mast cells. *Immunol Res* 2006;**34**:97–115.
5. Guo CB, Kagey-Sobotka A, Lichtenstein LM, Bochner BS. Immunophenotyping and functional analysis of purified human uterine mast cells. *Blood* 1992;**79**:708–12.
6. Woidacki K, Popovic M, Metz M, Schumacher A, Linzke N, Teles A, et al. Mast cells rescue implantation defects caused by c-kit deficiency. *Cell Death Dis* 2013;**4**:e462.
7. Meyer N, Woidacki K, Knofler M, Meinhardt G, Nowak D, Velicky P, et al. Chymase-producing cells of the innate immune system are required for decidual vascular remodeling and fetal growth. *Sci Rep* 2017;**7**:45106.
8. Woidacki K, Jensen F, Zenclussen AC. Mast cells as novel mediators of reproductive processes. *Front Immunol* 2013;**4**:29.
9. Meyer N, Woidacki K, Maurer M, Zenclussen AC. Safeguarding of fetal growth by mast cells and natural killer cells: deficiency of one is counterbalanced by the other. *Front Immunol* 2017;**8**:711.
10. Scanduzzi L, Beghdadi W, Daugas E, Abrink M, Tiwari N, Brochetta C, et al. Mouse mast cell protease-4 deteriorates renal function by contributing to inflammation and fibrosis in immune complex-mediated glomerulonephritis. *J Immunol* 2010;**185**:624–33.
11. Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* 2007;**217**:141–54.
12. Menzies FM, Higgins CA, Shepherd MC, Nibbs RJ, Nelson SM. Mast cells reside in myometrium and cervix, but are dispensable in mice for successful pregnancy and labor. *Immunol Cell Biol* 2012;**90**:321–9.
13. Lee H, Kashiwakura J, Matsuda A, Watanabe Y, Sakamoto-Sasaki T, Matsumoto K, et al. Activation of human synovial mast cells from rheumatoid arthritis or osteoarthritis patients in response to aggregated IgG through Fcγ receptor I and Fcγ receptor II. *Arthritis Rheum* 2013;**65**:109–19.
14. Kajiwara N, Sasaki T, Bradding P, Cruse G, Sagara H, Ohmori K, et al. Activation of human mast cells through the platelet-activating factor receptor. *J Allergy Clin Immunol* 2010;**125**:1137–45. e6.
15. Okumura S, Kashiwakura J, Tomita H, Matsumoto K, Nakajima T, Saito H, et al. Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcεRI. *Blood* 2003;**102**:2547–54.
16. Fujisawa D, Kashiwakura J, Kita H, Kikukawa Y, Fujitani Y, Sasaki-Sakamoto T, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol* 2014;**134**:622–33. e9.
17. Church MK, Benyon RC, Rees PH, Lowman MA, Campbell AM, Robinson C, et al. Functional heterogeneity of human mast cells. In: Galli SJ, Austen KF, editors. *Mast Cell and Basophil Differentiation and Function in Health and Disease*. New York: Raven Press; 1989. p. 161–70.
18. Woidacki K, Meyer N, Schumacher A, Goldschmidt A, Maurer M, Zenclussen AC. Transfer of regulatory T cells into abortion-prone mice promotes the expansion of uterine mast cells and normalizes early pregnancy angiogenesis. *Sci Rep* 2015;**5**:13938.
19. Croft M. Control of immunity by the TNFR-related molecule OX40 (CD134). *Annu Rev Immunol* 2010;**28**:57–78.
20. Lu LF, Lind EF, Gondek DC, Bennett KA, Gleeson MW, Pino-Lagos K, et al. Mast cells are essential intermediaries in regulatory T-cell tolerance. *Nature* 2006;**442**:997–1002.
21. Jones TG, Finkelman FD, Austen KF, Gurish MF. T regulatory cells control antigen-induced recruitment of mast cell progenitors to the lungs of C57BL/6 mice. *J Immunol* 2010;**185**:1804–11.