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Original Article

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

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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 decidua-derived MCs were generated by culturing decidual cells with stem cell factor. An ultrastructural analysis of primary decidual MCs and cultured decidua-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 decidua-derived MCs showed an FcεRIα$^+$Kit$^+$ tryptase$^+$chymase$^+$ phenotype. Their granules contenting particles exhibited variable amounts of electron-lucent space separating electron-dense particles. Both enzymatically dispersed decidual MCs and cultured decidua-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 decidua-derived MCs may enable the roles of decidual MC during pregnancy to be explored.

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

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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-FcεRIα 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 decidua-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 decidua-derived MCs

Human cultured decidua-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 Teknika, 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% ± 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

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