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Development of peritoneal macrophage along a dendritic cell lineage in response to uptake of oligomannose-coated liposomes

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ABSTRACT

In this study, we investigate the potential of peritoneal macrophages to differentiate into dendritic cell (DCs) in response to preferential uptake of oligomannose-coated liposomes (OMLs). About 30% of peritoneal cells (PECs) preferentially took up OMLs that were administered into the peritoneal cavity. The OML-ingesting cells expressed CD11b and F4/80, but lacked CD11c expression, indicating that the OML-ingesting PECs with a $CD11b^{high}CD11c^{-}$ phenotype are resident peritoneal macrophages. During *in vitro* cultivation, $CD11c^{+}$ cells arose among the PECs with ingested OMLs. $CD11c^{+}$ cells also developed among enriched peritoneal $CD11b^{high}CD11^{-}$ cells from OML-treated mice, and the resulting $CD11c^{+}$ cells expressed co-stimulatory molecules and MHC class II. In addition, OML-ingesting $CD11b^{high}CD11c^{+}$ cells were found in spleen after the enriched peritoneal macrophages with ingested OMLs were transplanted in the peritoneal cavity of mice. These results show that a fraction of peritoneal macrophages can differentiate into mature DCs following uptake of OMLs.

1. Introduction

Dendritic cells (DC) are potent professional antigen-presenting cells (APCs) that capture antigens and migrate to T cell regions of draining lymph nodes, where they mature into functional DCs to initiate primary immune responses [1–4]. Therefore, strategies that target DCs and modulate DC function *in vivo* may have significant implications for vaccine design [5,6].

We have demonstrated that liposomes coated with a neoglycolipid constructed from mannotriose and dipalmitoylphosphatidylethanolamine (Man3-DPPE) (oligomannose-coated liposomes, OMLs) induce a strong T helper 1 (Th1) immune response against encapsulated antigens in mice, with significant IFN-γ production and suppression of IL-4 production following preferential uptake of OMLs by peritoneal $CD11b^{high}$ cells [7,8]. In addition, intraperitoneal or subcutaneous administration of OMLs protects against subsequent protozoan infections and tumor inoculation when specific antigens are entrapped within the OMLs [9–12]. We also showed that OML uptake leads to upregulation of expression of costimulatory molecules and MHC class II molecules on the peritoneal $CD11b^{high}$ cells, with enhanced production of IL-12 [7], which is essential for induction of a Th1 immune response [13]. We also found that the peritoneal $CD11b^{high}$ cells effectively present encased antigen-derived peptides via MHC class I and II molecules and activate both $CD4^{+}$ and $CD8^{+}$ T cells in response to OML uptake [8]. These results indicate that OMLs can be used both as a specific antigen delivery vehicle to APCs and as an APC-activating adjuvant to induce strong cellular immunity. The peritoneal $CD11b^{high}$ cells also express high levels of F4/80, indicating that these cells can be classified as resting resident peritoneal macrophages, and these cells are the most potent target APCs in the peritoneal cavity for induction of OML-dependent immune responses [8]. On the other hand, it is well accepted that DCs, and not macrophages, are the most potent APCs that capture antigens at peripheral tissues, migrate to T cell regions of draining lymph nodes, and present antigens to initiate immune responses [1–4]. Therefore, to understand why OMLs induce specific immune responses after peritoneal administration requires clarification of how resting resident peritoneal macrophages can act as professional APCs after uptake of OMLs.

Cells of a monocyte/macrophage lineage can be directed to develop into potent immunostimulatory DCs in human when cultured in the presence of GM-CSF and IL-4 [14,15]. Randolph et al. showed that human and mouse monocytes differentiate into DCs on uptake of zymosan or microspheres with reverse transendothelial migration [16,17], and Rezzani et al. found that resting resident peritoneal macrophages obtained from peritoneal lavage can differentiate into DCs upon treatment with GM-CSF [18,19]. A peritoneal pool of $CD14^{+}$ mononuclear cells in humans can also differentiate into DCs or macrophages *in vitro* under appropriate conditions [20]. These findings prompted us to investigate the potential of resident peritoneal macrophages to differentiate into a

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DC lineage in response to OML uptake. In the present study, we show that peritoneal cells that belong to a monocyte/macrophage lineage become mature DC-like cells in response to OML uptake.

2. Materials and methods

2.1. Antibodies and reagents

Fluoroscein isothiocyanate (FTIC)- or phycoerythrin (PE)-labeled antibodies directed against mouse CD3ε, CD14, CD19, CD40, CD86, CCR7, F4/80, B220, and I-A/I-E class II molecules were purchased from eBioscience (Boston, MA, USA). A peridinin chlorophyll protein-cyanine 5.5 (PerCP-Cy5.5)-labeled anti-CD11b monoclonal antibody (mAb), an allophycocyanin (APC)-labeled CD11c mAb, and Fc-block (anti-mouse CD16/32) were purchased from BD PharMingen (San Diego, CA, USA). Isotype control antibodies were purchased from R&D Systems (Minneapolis, MN, USA).

2.2. Preparation of oligomannose-coated liposomes (OMLs)

Neoglycolipids constructed from mannotriose (Man3; Manα1-6(Manα1-3)Man) and dipalmitoylphosphatidylethanolamine (DPPE) were synthesized and purified in our laboratory and coated liposomes were prepared, as described previously [9,10]. Briefly, a chloroform–methanol (2:1, v/v) solution containing 1.5 μmol of DPPC, 1.5 μmol of cholesterol, and 0.15 μmol of neoglycolipid were added to a flask and evaporated to prepare a lipid film containing neoglycolipid. PBS (200 μl), 5 mg/ml of FITC-BSA containing PBS, or 5 mg/ml of ovalbumin (OVA) containing PBS was added to the dried lipid film and multilamellar vesicles were prepared by intense vortex dispersion. The vesicles were extruded 10 times through a 1-μm pore polycarbonate membrane (Nucleopore, Pleasanton, CA, USA). The amount of entrapped protein was measured using a modified Lowry protein assay kit (Pierce, Rockford, IL) in the presence of 0.3% (w/v) sodium dodecyl sulfate, using BSA as the standard. Molar ratios of the lipid components of the liposomes were determined using HPLC. Particle sizes of liposomes were determined by dynamic light scattering using a particle size analyzer (LB-550, Horiba, Kyoto, Japan).

2.3. Preparation and culture of peritoneal cells (PECs)

Six- to 10-week-old female C57BL/6 mice were purchased from Shizuoka Laboratory Animal Corporation (Hamamatsu, Japan). All animal experiments were conducted in compliance with the ethical requirements of the Animal Committee at Tokai University. A PBS suspension (200 μl) of OMLs or uncoated liposomes (60 μg of cholesterol) with or without encased FITC-labeled bovine serum

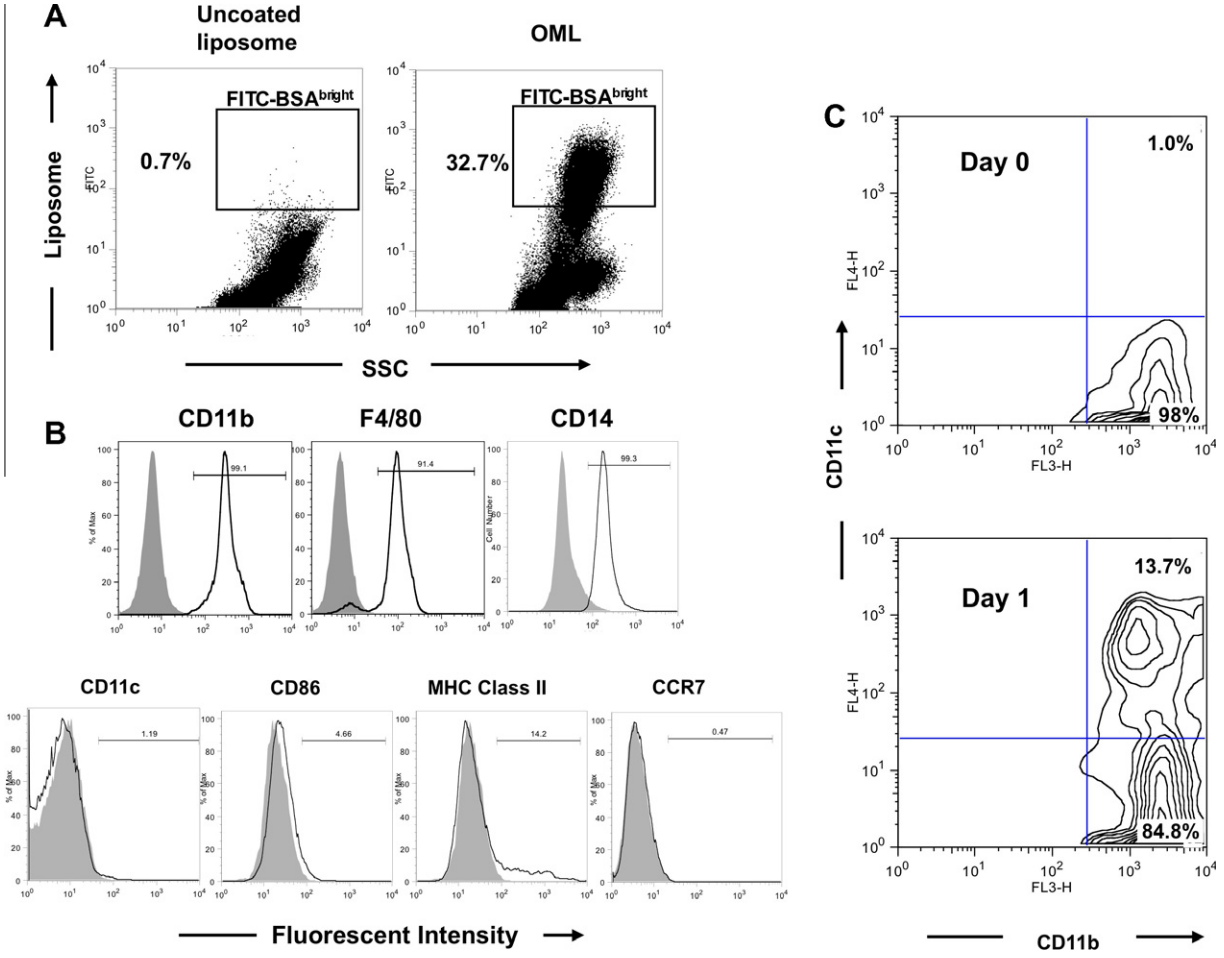

Fig. 1. Phenotypic analysis and CD11c expression of PECs with ingested OMLs. OMLs or uncoated liposomes with encased FITC-BSA were injected into the peritoneal cavity. PECs were recovered 1 h after injection and stained with PerCP-Cy5.5-labeled anti-CD11b mAb and PE- or APC -labeled mAbs against mouse CD11c, CD14, CD86, CCR7, F4/80 and MHC class II. The expression of these molecules on PECs was analyzed by flow cytometry. (A) Incorporation of liposomes in the peritoneal cells. (B) Expression of hematopoietic cell lineage markers in the cells with ingested OMLs. Peritoneal cells with incorporated liposomes were gated based on the FITC fluorescence (indicated in the square in panel A), and expression of other molecules in the gated cells was analyzed. Gray solid peaks and open peaks indicate cells treated with isotype control and specific antibodies, respectively. (C) Expression of CD11b and CD11c in the gated cells obtained from OML-treated mice was analyzed before and after *in vitro* cultivation for 1 day. The data are representative of those obtained in three separate experiments.