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Signaling through Toll-like receptor 3 and Dectin-1 potentiates the capability of human monocyte-derived dendritic cells to promote T-helper 1 and T-helper 17 immune responses

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Abstract

Background aims. Recent studies have shown that the ligation of Toll-like receptor 3 (TLR3) or Dectin-1 on human monocyte-derived dendritic cells (MoDC) elicits their maturation, but with a different outcome on immunomodulation. Therefore the aim of this work was to study the response of MoDC to the combined effect of polyinosinic:polycytydilic acid [Poly (I:C)] and curdlan, selective TLR3 and Dectin-1 agonists, respectively. *Methods.* Immature MoDC, generated from human monocytes, were treated with Poly (I:C), curdlan or their combination for 2 days. Phenotypic characteristics of MoDC were determined by flow cytometry, and cytokine production was measured by enzyme-linked immunosorbent assay (ELISA) and FlowCytomix, while the stimulatory capability of MoDC was tested using a mixed leukocyte reaction assay. *Results.* The combination of Poly (I:C) and curdlan induced phenotypic maturation of MoDC with the capability to stimulate an alloreactive response. Such treated MoDC up-regulated the production of interleukin (IL)-12, IL-23 and IL-10, compared with the effect of Poly (I:C) alone. Curdlan-treated MoDC stimulated the production of IL-17 by alloreactive CD4 + T cells more strongly than Poly (I:C)-treated MoDC. The opposite effect was observed for interferon (IFN)-γ production. When combined, these agonists primed MoDC to increase further the production of IFN-γ by CD4 + T cells in co-culture, especially those of naive (CD45RA +) phenotype, and IL-17 by memory (CD45RO +) CD4 + T cells. *Conclusions.* Ligation of TLR3 and Dectin-1 receptor up-regulates T-helper (Th) 1 and Th17 immune responses compared with single agonists. These findings may have therapeutic implications for the use of MoDC in immunotherapy.

Key Words: *Dectin-1 receptor, human monocyte-derived dendritic cells, T-helper immune response, Toll-like receptor 3*

Introduction

Dendritic cells (DC), when activated by antigens of infectious micro-organisms and inflammatory products, are mobile sentinels that capture, process and bring antigens to T cells. DC migrate from the periphery to lymphoid organs, express co-stimulatory molecules, and secrete cytokines for the induction of the immune response (1). These potent antigen-presenting cells (APC) express a wide variety of pattern recognition receptors (PRR) by which they recognize a conserved groups of molecules collectively known as pathogen-associated molecular patterns (PAMP). Although triggering of a single PRR, especially Toll-like receptors (TLR) or C-type lectins, results in phenotypic changes in DC, for functional maturation co-operation between multiple PRR is needed in order to achieve an effective immune response (2).

DC-based cancer vaccines have provided encouraging results (3). Human monocyte-derived DC (MoDC) can be prepared easily by plastic adherence of monocytes from peripheral blood mononuclear cells (PBMC) followed by incubation for 5–7 days in granulocyte–monocyte colony-stimulating factor (GM-CSF) and interleukin (IL)-4 containing medium (4). It has been established that maturation of DC is essential because mature DC have a more stable phenotype and are superior stimulators of T-cell responses (5). Therefore a major effort has been made to identify an optimal protocol for DC maturation.

The current 'gold standard' for the generation of DC used in DC-based cancer vaccine studies is maturation of MoDC with tumor necrosis factor (TNF)-α, IL-1β, IL-6 and prostaglandin E_2 (PGE_2)

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(6). One weakness with DC generated in this way is the absence of IL-12 secretion, a key cytokine for induction of effective T-helper (Th) 1 and cytotoxic T-lymphocyte (CTL) responses that are assumed to be essential for cancer vaccination therapy (7).

The combined activation of different PRR can result in complementary, synergistic or antagonistic effects that modulate innate and adaptive immunity (8). Polyinosinic:polycytidylic acid [Poly (I:C)], a synthetic analog of dsRNA and a Toll-like receptor 3 (TLR3) agonist, has been reported to induce stable mature Th1 responses, promoting clinically applicable DC that produce large amounts of IL-12 (9). Dectin-1, a DC-associated C-type lectin, is the first of many PRR that mediate their own signaling and induce the maturation of DC capable of eliciting the generation of different Th effectors. The treatment of DC with Dectin-1 agonists results in their maturation with the capability of eliciting differentiation of Th1 and Th17 cells (10–12).

In order to identify a maturation cocktail combining a high yield of DC, potent expression of maturation markers, co-stimulatory and adhesion molecules together with a high production of Th cytokines crucial for the anti-tumor immune response, we co-stimulated MoDC with a combination of Poly (I:C) and curdlan, a ligand for Dectin-1 receptor. We show that the combination is superior in triggering Th1 and Th17 responses compared with the effect of individual agonists.

Methods

Medium and reagents

Human MoDC were cultured in complete RPMI-1640 medium (ICN, Costa Mesa, CA, USA), composed of 2 mM L-glutamine, 20 μg/mL gentamicin, 50 μM 2-mercaptoethanol (2-ME; Sigma-Aldrich, Munich, Germany) and 10% heat-inactivated fetal calf serum (FCS; Invitrogen, Carlsbad, CA, USA). Recombinant human IL-4 was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Recombinant human GM-CSF (Leucomax, specific activity 4.44×10^6 UI) was obtained from Schering-Plough (Basel, Switzerland). Curdlan from *Alcaligenes faecalis* and Poly (I:C) were from Sigma-Aldrich. Monensin sodium was obtained from Sigma-Aldrich.

Cell preparation and MoDC cultures

MoDC were generated from PBMC. Briefly, PBMC from buffy coats of six healthy volunteers (upon written informed consent) were isolated by density centrifugation on Lymphoprep (Nycomed, Oslo, Norway), resuspended in 5 mL 10% FCS with 2-ME in RPMI medium and allowed to adhere to plastic flasks. After 2 h at 37°C, non-adherent cells were removed and adherent cells were cultured in 5 mL complete RPMI medium containing GM-CSF (100 ng/mL) and IL-4 (20 ng/mL). At day 3, a half-of-medium volume was removed and replaced by the same volume of fresh medium containing GM-CSF and IL-4. After 6 days MoDC were replated (5×10^5 cells/mL) in medium with GM-CSF/IL-4 and the addition of different concentrations of curdlan, Poly (I:C) or their combination for an additional 2 days. After 8 days cell-free supernatants were collected and stored at −20°C for subsequent determination of cytokine levels and the cells were used for immunologic studies.

Allogeneic T-cell activation

The ability of $CD4^+$ T cells or their subsets to proliferate was tested in an allogeneic mixed leukocyte reaction (MLR). Allogeneic $CD4^+$ T cells were isolated from PBMC using negative immunomagnetic sorting with $CD4^+$ isolation kits (MACS technology; Myltenyi Biotec, Bergish Gladbach, Germany) following the instructions of the manufacturer. The sorted $CD4^+$ T cells were separated further using a $CD4^+$ CD45RA kit (MACS technology; Myltenyi Biotec). $CD4^+$ $CD45RA^+$ and $CD4^+$ $CD45RO^+$ cells were recovered in positive and negative fractions, respectively. Their purity was higher than 95% as checked by using specific fluorochrome-labeled monoclonal antibodies (MAb) and flow cytometry.

Purified $CD4^+$ T cells, $CD4^+$ $CD45RO^+$ T cells or $CD4^+$ $CD45RA^+$ T cells (1×10^5 cells/well) were cultivated with different numbers of allogeneic MoDC in complete RPMI medium with 10% FCS in 96-well round-bottomed cell culture plates. Different MoDC:$CD4^+$ T-cells ratios were used. After 5 days of culture, the cells were pulsed with [^{3}H]thymidine for the last 18 h (1 μCi/well; Amersham, Amersham, UK). Labeled cells were harvested onto glass fiber filters and the incorporation of the radionuclide into DNA was measured further by β-scintillation counting (LKB-1219; Rackbeta, Turku, Finland). Results were expressed as counts per minute (c.p.m.) ± standard deviation (SD) of triplicates.

Flow cytometry

Control and stimulated MoDC (2×10^5 cells/tube) were washed in phosphate-buffered saline (PBS) supplemented with 2% FCS and 0.1% NaN_3, and incubated for 45 min at 4°C with one of the following MAb: anti-HLA-DR coupled with phycoerythrin (PE), CD80 conjugated with fluorescein isothyocianate (FITC), CD83–FITC, CD86–PE, CD40–FITC (Serotec, Oxford, UK), CD54–PE (Serotec) and CCR7–FITC (R&D Systems, Minneapolis, MN,