Passive induction of experimental autoimmune encephalomyelitis by activated or tolerogenic dendritic cells

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Method Article

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Abstract

Experimental autoimmune encephalomyelitis \(EAE) is a widely accepted murine model for studying pathological features of multiple sclerosis \(MS). Induction of EAE results infiltration of T cells and demyelination of axon in central nervous system, further leading the clinical symptoms of MS. Beside T cells, dendritic cells \(DCs) is a key importance for initiation of EAE through their antigen presentation ability. Because of the plasticity of DCs, agents \(CDDO-DFPA in our lab) that repress activated DCs confer a tolerogenic DC \(TolDC) phenotype. TolDCs have emerged as clinical targets for the treatment of autoimmunity. This protocol describes a method for passive induction of EAE by adoptive transfer of DCs primed with myelin antigen into naïve mice. We have shown the ability of inducing EAE between activated DCs and TolDCs in associated publication “A unique tolerizing dendritic cell phenotype induced by the synthetic triterpenoid CDDO-DFPA \(RTA-408) is protective against EAE” in _Scientific report._

Introduction

Both MS and EAE are characterized by the infiltration of inflammatory cells, including T cells, B cells, macrophages and DCs and demyelination of axonal tracks in the central nervous system.[1] EAE can be initiated by 1. active induction: immunization with myelin antigens in adjuvant[2] or 2. passive induction: adoptive transfer of myelin-specific T cells.[3] Both methods depend on the same fundamental principles. Beside T cells, evidences from clinical researches and basic experimental models indicate that DCs are involved in the pathogenesis of most autoimmune diseases.[4] In addition, among the antigen-presenting cells \(APCs), DCs alone are sufficient to prime myelin-reactive T cells to induce EAE.[5] Therefore, due to plasticity of DCs, agents that alter the DC differentiation from activation to tolerance, have been emerged and applied on the clinical trials.[6-8] This results TolDCs to become a potential therapy in the clinical treatment of autoimmune disorders.[9-11] In order to test the function of agents in inducing TolDC phenotype, the protocol has been established to show the passive induction of EAE by adoptive transfer of DCs primed with myelin antigen into naïve mice. Hematopoietic stem cells \(HSCs) were isolated from mice bone marrow and cultured with differentiation factors to generate bone marrow-derived DCs \(BMDCs). BMDCs were treated with LPS for activation before received agents \(CDDO-DFPA in our publication) for tolerogenic phenotypes or PBS. BMDCs were subcutaneously injected into C57BL/6 mice once each week for four consecutive weeks. Mice were monitored daily symptoms of EAE, using standard criteria \(**Figure 1**).[12]

Reagents

GM-CSF \(Peprotech Inc.) IL-4 \(Peprotech Inc.) lipopolysaccharides \(LPS) \(Sigma Aldrich Inc.) β-mercaptoethanol \(Sigma Aldrich Inc.) pertussis toxin \(PTX) \(BD bioscience) MOG \(35-55) peptide \(21stCentury Biochemicals) ACK buffer \(Gibco) RPMI-1640 plus L-glutamine \(Gibco) FBS \(premium select, low endotoxin) \(Atlanta biologicals) Penicillin/streptomycin \(Gibco) CD11c antibody for Flow cytometry \(Becton Dickinson & Co.)

Equipment

Basic cell culture equipment Dissecting tools 3 ml syringe with 21 gauge \(Becton Dickinson & Co.) Centrifuge with temperature control Cell strainer \(40 µm) Cell incubator Flow cytometry 1 ml syringe with 26 gauge \(Becton Dickinson & Co.)

Procedure

Preparation and characterization of bone marrow-derived dendritic cells Bone marrow-derived dendritic cells \(BMDCs) were expanded from hematopoietic progenitors isolated from C57BL/6 mice.[13] After red cell lysis with ACK buffer, progenitors were cultured in RPMI-1640 plus L-glutamine, 10% FBS, 50 nM β-mercaptoethanol, and 5% penicillin/streptomycin. Fresh medium with 15 ng/ml of GM-CSF and 10 ng/ml of IL-4 were added to cultures on day 0, 3, and 5. BMDCs were harvested on day 7 and analyzed by flow cytometry for CD11c expression \(70-80% of the expanded cell population, **Figure 2**). **Preparation of mature or tolerogenic DCs** BMDCs were harvested on day 7 and pre-treated with the absence or presence of CDDO-DFPA \(400 nM) for 4 hours before LPS \(100 n□g/ml) treatment for 24 hours. BMDCs were then treated with MOG \(35-55) \(100 µg/ml) for 4 hours prior to the administration. **Induction and evaluation of EAE** EAE was induced in 8-10 week old female C57BL/6 mice. Methods for passive induction of EAE by ex vivo expanded BMDCs were adopted from previously described protocol.[14] In brief, mice received 2×10^6 LPS-stimulated, MOG-pulsed CD11c+ BMDCs at a total volume of 200 µ□L injected subcutaneously \(100µ □L into each hind leg). On the day of BMDC transfer and 48 hours later, each mouse also received 200 ng of pertussis toxin \(PTX), and DCs were injected into C57BL/6 mice once each week for four consecutive weeks. Mice were monitored daily for survival and symptoms of EAE, using standard criteria \(**Video 1**).[12]

Timing

Preparation and characterization of bone marrow-derived dendritic cells- 7 days **Preparation of matured or tolerogenic DCs**- 1 days **Induction of EAE**- 4 weeks **Evaluation of EAE**- 35 days

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Figures

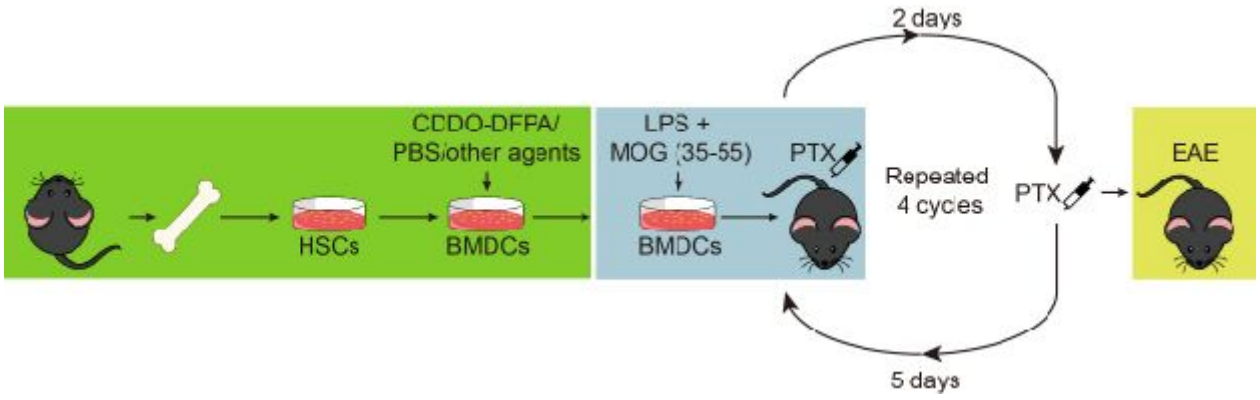

Figure 1

Flow chart

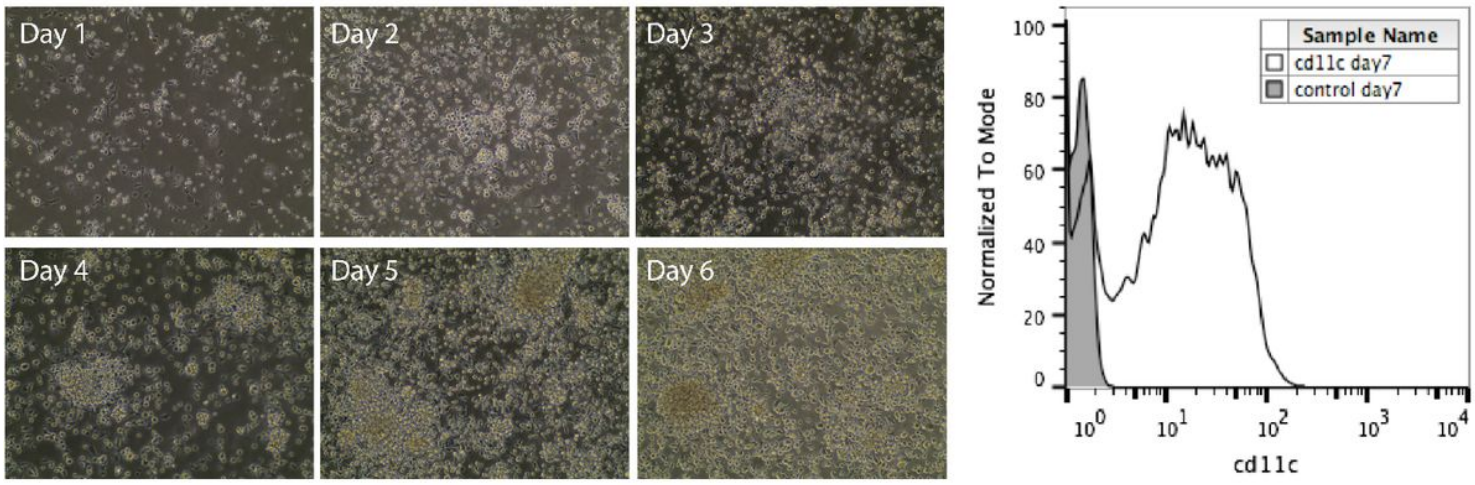

Figure 2

Preparation and characterization of BMDCs

Supplementary Files

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