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Short communication

The neurotrophic hepatocyte growth factor induces protolerogenic human dendritic cells

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ABSTRACT

Hepatocyte growth factor (HGF) limits mouse autoimmune neuroinflammation by promoting the development of tolerogenic dendritic cells (DCs). Given the role played by DCs in the establishment of immunological tolerance, agents that coerce DCs to adopt a protolerogenic function are currently under investigation for multiple sclerosis (MS) therapy. Here, we studied the immunomodulatory effects of HGF on DCs derived from human monocytes. DCs differentiated in the presence of HGF adopt a protolerogenic phenotype with increased ability to generate regulatory T cells, a property that might be exploited therapeutically in T cell-mediated immune disorders such as MS.

1. Introduction

The recognition of multiple sclerosis (MS) as an immune-mediated inflammatory demyelinating neurodegenerative disorder (Sospedra and Martin, 2005) imparts the need to develop novel therapeutic strategies that could cripple the three hallmarks of MS simultaneously (Hemmer and Hartung, 2007). This can be accomplished with the power of combinatorial or single-agent therapy eliciting multiple protective and reparative processes simultaneously. In the search for single new therapeutics combining these requirements, recent findings indicate that hepatocyte growth factor (HGF) is one such candidate (Benkhoucha et al., 2010, Bai et al., 2012).

HGF is a factor with strong neuroprotective properties (Ebens et al., 1996) reported to enhance the migration and differentiation of myelin producing oligodendrocyte precursor cells (Lalive et al., 2005, Kitamura et al., 2007). In animal models of MS, treatment with HGF results in functional improvement that reflects both modulation of the immune response and myelin repair (Benkhoucha et al., 2010, Bai et al., 2012). Data from MS patients further suggest that HGF may contribute to both stimulation for remyelination (Muller et al., 2012) and immune modulation (Molnarfi et al., 2012).

While DCs play a major role in the initiation of T cell-mediated CNS autoimmunity (Steinman and Banchereau, 2007), DCs alternatively may favor the induction of tolerance, and thus make the induction of immunological tolerance by DCs an attractive strategy in MS (Raiotach-Regue et al., 2012, Nuyts et al., 2013). The administration of tolerogenic immature antigen-pulsed monocyte-derived DCs (MoDCs) in healthy volunteers has shown great potential (Dhodapkar et al., 2001) and augur well the future use of these cells for clinical application.

Here we report that immature human MoDCs differentiated in the presence of HGF exhibited lower expression of HLA-DR and co-stimulatory molecules and an enhanced capacity to generate responding T cells with a regulatory phenotype, compared with conventional immature MoDCs. Our findings further provide a proof of concept for future application of HGF in MS therapy.

2. Materials and methods

2.1. Standard protocol approvals

Peripheral blood monocytes were isolated from buffy coats of blood of healthy volunteers (eleven male and four female, median age 45 years, range 19–67 years) provided by the Geneva Hospital Blood Transfusion Center. In accordance with the ethical committee of the Geneva Hospital, the blood bank obtained informed consent from the

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Table 1
HGF supplementation does not interfere with MoDC development.

	CD1a	CD1c	CD14	CD11c	CD209
Monocytes	1.6 (0.2–2.4)	8.6 (2.2–14.4)	74.0 (69.7–80.5)	5.7 (0.0–16.4)	0.6 (0.0–2.1)
Ctr-MoDCs	90.2 (86.9–92.2)	98.3 (97.2–99.3)	3.4 (0.5–8.6)	97.7 (97.4–98.0)	95.9 (94.7–97.0)
HGF-MoDCs	91.9 (87.7–92.3)	97.5 (95.4–99.5)	1.7 (0.4–4.0)	97.6 (97.1–98.1)	96.2 (95.3–96.6)

The data indicate the median (min–max) % of three independent experiments.

donors, who are thus informed that part of their blood will be used for research purposes.

2.2. Cytokines and reagents

Human recombinant GM-CSF and IL-4 were obtained from Miltenyi Biotec. Human recombinant HGF was obtained from eBioscience. LPS was obtained from Sigma (*Escherichia coli* 055:B5).

2.3. Generation of DCs from peripheral blood monocytes

Highly purified $CD14^+$ monocytes were isolated from the mononuclear fraction using negative selection microbeads (Human Monocyte Enrichment Kit; EasySep, STEMCELL Technologies). Monocytes were cultured for 6 days at 2.0×10^6 per ml in 6-well tissue culture plates (Falcon, Becton-Dickinson) in standard culture medium consisting of RPMI 1640 medium with 10% fetal bovine serum (Thermo Fisher Scientific) supplemented every second day with 20 ng/ml GM-CSF and 20 ng/ml IL-4 (Ctr-MoDCs); 20 ng/ml GM-CSF and 20 ng/ml IL-4 and 30 ng/ml HGF (HGF-MoDCs). After six days in culture, the non-adherent cells were recovered for use as immature MoDCs in subsequent assays. Day 6 immature MoDCs were matured by using 5 ng/ml of LPS for 24 h.

2.4. T-cell isolation, mixed lymphocyte reaction (MLR)

MLR was performed using purified allogeneic T cells as responder cells and MoDCs as stimulator cells. Highly purified $CD4^+$ T cells were isolated from PBMCs by negative selection (Human $CD4^+$ T Cell Enrichment Kit; EasySep, STEMCELL Technologies). MoDCs (10×10^3) were cultured for 7 days with allogeneic responder $CD4^+$ T cells (10×10^4) in 96-well flat-bottom microtest plates (Costar, Integra Biosciences).

2.5. Immunologic markers and flow cytometry

MoDC staining was performed using fluorochrome-conjugated antibodies or with the appropriate fluorochrome-conjugated, isotype-matched irrelevant mAbs from the same provider to establish background fluorescence. Expression of FoxP3 was detected in fixed/permeabilized $CD3^+CD4^+CD25^+$ (BioLegend) T cells using the Anti-Human FoxP3 Staining Kit (eBioscience). Samples were run through a FACSCanto flow cytometer (Becton Dickinson) with standard equipment.

2.6. Analysis of cytokine production

Cell-free supernatants were analyzed (Bioplex, Biorad) for cytokine content using a multiplex bead-based assay (Luminex Performance assay; R&D Systems) according to the manufacturer's instructions. The results are expressed as an average of triplicate wells ± standard error of the mean (SEM).

2.7. Statistical analysis

Comparisons were performed by Student's *t* test. Values of $p < 0.05$ were considered statistically significant.

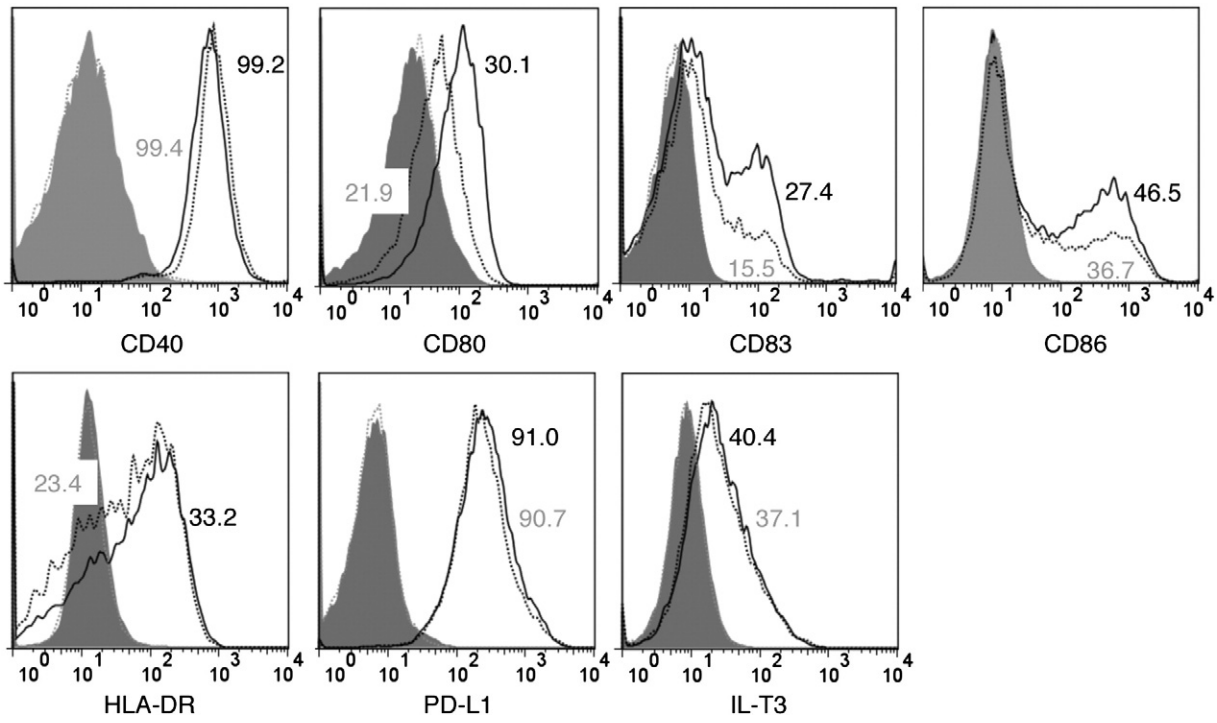

Fig. 1. HGF decreases HLA-DR, CD80, CD83 and CD86 expression by immature MoDCs. MoDCs were generated from monocytes cultured with GM-CSF and IL-4 for 6 days. Expression of HLA-DR, CD40, CD80, CD83, CD86, PD-L1, and IL-T3 by control MoDCs and MoDCs differentiated in the presence of 30 ng/ml HGF was determined by FACS analysis. HGF decreased the expression of HLA-DR molecules. Overlapping histogram profiles of the expression of each marker. Data are representative of three independent experiments. Ctr-MoDCs are represented by a thick solid line, and HGF-MoDCs are represented by a thick dotted line. MoDCs stained with isotype matched mAb are represented by a gray profile.