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Ageing impairs the T cell response to dendritic cells

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ABSTRACT

Dendritic cells (DCs) are critical in priming adaptive T-cell responses, but the effects of ageing on interactions between DCs and T cells are unclear. This study investigated the influence of ageing on the maturation of and cytokine production by human blood-enriched DCs, and the impact on T cell responses in an allogeneic mixed leucocyte reaction (MLR). DCs from old subjects (65–75 y) produced significantly less TNF-α and IFN-γ than young subjects (20–30 y) in response to lipopolysaccharide (LPS), but expression of maturation markers and co-stimulatory molecules was preserved. In the MLR, DCs from older subjects induced significantly restricted proliferation of young T cells, activation of $CD8^+$ T cells and expression of IL-12 and IFN-γ in T cells compared with young DCs. T cells from older subjects responded more weakly to DC stimulation compared with young T cells, regardless of whether the DCs were derived from young or older subjects. In conclusion, the capacity of DCs to induce T cell activation is significantly impaired by ageing.

Introduction

Immunosenescence involves a progressive decline in immune function, which is associated with increased susceptibility to infections and poor response to vaccination. Many aspects of adaptive immunity are affected by ageing (Dunn-Walters et al. 2003; Swain et al. 2005; Frasca et al. 2008). DCs play a central role in the priming of adaptive immune responses, but the influence of ageing on DC function remains poorly understood. Some studies demonstrate reduced numbers of both plasmacytoid and myeloid DCs (pDCs and mDCs) in the peripheral blood of older subjects (Shodell and Siegal 2002; Della Bella et al. 2007; Perez-Cabezas et al. 2007), but there is inconsistent data regarding the effects of ageing on DC phenotype and the ability of DCs to function as antigen presenting cells (Agrawal and Gupta 2011; Solana et al. 2012).

Induction of T cell responses by DCs involves the binding of antigen with major histocompatibility complex, DC expression of surface co-stimulatory molecules (e.g. CD40, CD80 and CD86), interaction with ligands on the T cell surface and production of cytokines. The efficacy of DCs in inducing T cell responses depends on a number of factors; impaired maturation of DCs, or altered cytokine production are likely to have a knock-on effect on the T-cells that they stimulate. To date, data regarding the influence of ageing on human DC expression of co-stimulatory molecules and cytokine production, in response to *in vitro* maturation-inducing stimuli, has been inconsistent, showing either comparable or reduced DC function in the elderly (Lung et al. 2000; Pietschmann et al. 2000; Saurwein-Teissl et al. 2000; Shurin et al. 2007).

It is not clear whether DCs from young subjects are able to overcome the impaired responsiveness of T cells from older subjects and human studies investigating this are very limited (Agrawal et al. 2007a,b; Jiang et al. 2011). Lung et al. (2000) demonstrated that human monocyte-derived DCs from young and older subjects were equally effective in stimulating proliferation of and cytokine secretion by influenza-specific $CD4^+$ T cells. In contrast, Agrawal et al. (2007a,b) demonstrated that monocyte-derived DCs from older people were defective in stimulating proliferation of $CD4^+$ naïve T cells from young subjects.

The aim of the present study was to investigate the influence of ageing on the maturation of and cytokine production by human blood-enriched DCs, and to investigate whether these age-induced alterations have an impact on DC and T cell responses in the MLR. By avoiding the use of cell lines and long cultures with addition of cytokines and growth factors, this represents a novel approach to study the influence of ageing on both DC and T cell function.

Abbreviations: CCR, C-C chemokine receptor type; DCs, dendritic cells; FCS, foetal calf serum; FoxP3, forkhead box P3; LDC, low density cells; LPS, lipopolysaccharide; MLR, mixed leucocyte reaction; mDCs, myeloid DCs; PBMC, peripheral blood mononuclear cell; pDCs, plasmacytoid DCs; TLR, toll-like receptor; Treg, regulatory T cells.

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Materials and methods

Peripheral blood mononuclear cell (PBMC) preparation and culture

Peripheral blood was obtained from healthy young (20–30 y) and older (65–75 y) subjects. Exclusion criteria included diabetes requiring medication, asplenia and other acquired or congenital immunodeficiencies, any autoimmune disease, malignancy, cirrhosis, connective tissue diseases; current use of immunomodulating medication (including oral prednisone and inhaled steroids), self-reported symptoms of acute or recent infection, use of antibiotics within last 3 months, alcoholism and drug misuse (University of Reading Ethics Committee project ref 10/05). Blood was diluted into an equal volume of RPMI 1640 medium. PBMCs were isolated by density gradient centrifugation over Ficoll–Paque (Fisher Scientific, UK), and resuspended in RPMI 1640 medium with 10% foetal calf serum (FCS, Sigma Ltd., UK). PBMCs were cultured overnight in culture flasks (2×10^7 cells/flask) in a 37 °C, 5% CO_2 atmosphere.

Human blood DC enrichment and culture

Low density cells (LDCs) were prepared as the source of human blood-enriched DCs (which normally represent 1–2% of PBMCs). The LDCs had morphological characteristics of DCs, as described in previous studies (Knight et al. 1986; Kerdiles et al. 2010), typically 98–100% HLA-DR positive and stimulate strong proliferation of allogeneic T-cells at very low concentrations (Knight et al. 1986; Holden et al. 2008). Since DCs are unique in their ability to generate primary T-cell responses, T-cell responses generated in the MLR were interpreted as a result of DC stimulation. Unlike monocyte-derived DCs, homing markers are not altered during culture of LDCs, which is an advantage (Mann et al. 2012).

After overnight culture of PBMC, the non-adherent cells were collected and centrifuged over Nycoprep ($500 \times g$, 15 min) (PROGEN Biotechnik GmbH). LDCs were removed from the interface, washed twice ($650 \times g$, 5 min) in RPMI 1640 medium with 10% FCS and resuspended in the same medium.

LDCs were adjusted to a concentration of 1×10^6 cells/ml and cultured in the presence or absence of 10 μg/ml LPS in a 37 °C, 5% CO_2 atmosphere for 24 h (see Fig. 1).

T cell purification and DC stimulation of T cells

In this allogeneic MLR, T cells were obtained from blood donated by healthy young or older subjects, which were different from the DC donors. After isolating PBMCs, T cells were separated by negative isolation using a Human T cell enrichment kit (BD Bioscience, UK). The purity of T cells after this selection process was higher than 98%, as determined by CD3 antibody staining. Prior to culture, purified T cells were labelled with carboxyfluorescein diacetate succinimidyl ester (CFSE, Invitrogen Ltd., UK) for subsequent assessment of T cell proliferation. T cells (4×10^5) from young and older subjects were co-incubated with 3% cultured LDCs from young and older subjects in a 37 °C, 5% CO_2 atmosphere for 5d, using the experimental design illustrated in Fig. 1.

Intracellular cytokine production

Intracellular cytokine production by DCs following 24 h incubation in the presence or absence of LPS, or by T cells following the MLR was analysed using flow cytometry. Cells were incubated with or without 50 μl Monensin (3 μM) (eBioscience Ltd., UK) in a 37 °C, 5% CO_2 atmosphere for 4 h, washed in FACS buffer (BD Bioscience, UK) and stained with the appropriate surface marker antibodies before fixation, permeabilization and staining with appropriate antibodies for intracellular cytokines.

Antibody staining

For identification and characterization of peripheral blood DCs, cultured LDCs were stained with HLA-DR (APC, PerCP-Cy 5.5 or PE) and with a lineage cocktail containing antiCD3, CD14, CD19, CD20 (FITC). Cells that were negative to the cocktail but positive to HLA-DR were identified as DCs (see Fig. 2A). On average, 93% of LDCs were $Lin^-HLA\text{-}DR^+$. This was used in conjunction with antibodies for maturation markers (CD80 (PE-Cy 7), CD86 (APC) and CD40 (APC-Cy 7)), or a migration marker, C-C chemokine receptor type7 (CCR7) (PerCP-Cy 5.5). Cultured T cells were identified by CD3 (PE-Cy 7, APC or APC-Cy 7) staining (see Fig. 2B) and classified into CD4 (PE-Cy 7) and CD8 (PerCP-Cy 5.5) subsets. CD25 (APC) was used as a marker for T cell activation; and integrin β7 (PE) as a homing marker. Antibodies against IL-10 (PE), IL-12 (PE), TNF-α (PerCP-Cy 5.5), IFN-γ (APC-Cy 7) and TGF-β (PE-Cy 7) were used to assess intracellular cytokine production by $Lin^-HLA\text{-}DR^+$ DCs or DC-stimulated $CD3^+$ T cells. Isotype-matched control antibodies included rat IgG2a (PE), mouse IgG1 (FITC, PE, PE-Cy 7, PerCP-Cy 5.5, APC-Cy 7), mouse IgG2a (PerCP-Cy 5.5) and mouse IgG2b (PerCP-Cy 5.5). Stained cells were incubated at room temperature in the dark for 30–45 min, washed twice, resuspended in Fix solution and kept at 4 °C until analysis by flow cytometry. The lineage cocktail, CD80, HLA-DR (APC), CD3 (APC-Cy 7), CD25, IL-10, IL-12 and rat IgG2a were obtained from BD Biosciences, and all other antibodies were purchased from Cambridge Bioscience Ltd., UK.

Flow cytometric analysis

Samples were analysed using a FACSCanto II flow cytometer (BD, UK). Data were analysed by superenhanced Dmax (SED) normalized subtraction using FlowJo software.

Statistical analysis

Statistical analysis was performed using Mini Tab 16.0. Data were tested for normality and transformed using the Johnson Transformation where appropriate. Significant differences ($P < 0.05$) were evaluated by the Student's *t*-test or two-way ANOVA using the general linear model, followed by appropriate post hoc tests with Bonferroni correction. All data are shown as mean ± SE (standard error).

Results

Ageing impairs cytokine production by DCs, but not surface marker expression

DCs in LDC preparations from human blood were identified as $HLA\text{-}DR^+$ and negative for a lineage cocktail containing CD3, CD14, CD19 and CD20 antibodies (Fig. 2A). The proportion of DCs was similar in the young and older subjects. Cell surface expression of CD80, CD40 and CCR7, but not CD86, were significantly up-regulated after conditioning with LPS (Fig. 3). None of the surface markers, or their up-regulation by LPS, were affected by ageing (Fig. 3).

There were significant effects of ageing on LPS-induced production of TNF-α and IFN-γ by DCs (Fig. 4). LPS induced production of TNF-α and IFN-γ in DCs from the young subjects only; DCs from the older subjects were unresponsive (Fig. 4). It was notable that IFN-γ production by unstimulated DCs from older subjects was higher than those from young subjects (Fig. 4). LPS induced production of IL-10, IL-12 and TGF-β to a similar degree in young and older DCs data not shown.