

## SHORT COMMUNICATION

## Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection

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Dendritic cells (DCs) are key immune sentinels linking the innate and adaptive immune systems. DCs recognise danger signals and initiate T-cell tolerance, memory and polarisation. They are critical cells in responding to a viral illness. Obese individuals have been shown to have an impaired response to vaccinations against virally mediated conditions and to have an increased susceptibility to multi-organ failure in response to viral illness. We investigated if DCs are altered in an obese cohort (mean body mass index  $51.7 \pm 7.3 \text{ kg m}^{-2}$ ), ultimately resulting in differential T-cell responses. Circulating DCs were found to be significantly decreased in the obese compared with the lean cohort (0.82% vs 2.53%). Following Toll-like receptor stimulation, compared with lean controls, DCs generated from the obese cohort upregulated significantly less CD83 (40% vs 17% mean fluorescence intensity), a molecule implicated in the elicitation of T-cell responses, particularly viral responses. Obese DCs produced twofold more of the immunosuppressive cytokine interleukin (IL)-10 than lean controls, and in turn stimulated fourfold more IL-4-production from allogenic naive T cells. We conclude that obesity negatively impacts the ability of DCs to mature and elicit appropriate T-cell responses to a general stimulus. This may contribute to the increased susceptibility to viral infection observed in severe obesity.

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## INTRODUCTION

Obese persons are at risk of a diverse range of comorbid conditions such as type 2 diabetes, dyslipidaemia and cardiovascular disease. There is also an increasing body of literature describing chronic low-grade inflammation and significant immune dysregulation.<sup>1,2</sup> Impaired immune responses lead to an increased susceptibility to infection, although the underlying mechanisms remain unclear. Epidemiological studies have found increased rates of sepsis, pneumonia and wound infections in obese inpatients.<sup>3</sup> Obesity appears to confer an inadequate immune response to vaccination against viral conditions in mice<sup>4</sup> and humans,<sup>5,6</sup> and an increased susceptibility to overwhelming sepsis following viral illness.<sup>7,8</sup> Severe obesity was considered an independent risk factor for the severity and mortality of H1N1 influenza infection.<sup>7</sup>

Myeloid dendritic cells (DCs) have been described as the conductors of the immune system, orchestrating carefully phased responses to microbial, cancer and vaccine antigen stimuli. They reside as antigen-capturing cells in peripheral tissues and respond rapidly to inflammatory cytokines and microbial products. Once they have acquired and processed foreign antigens, they initiate an adaptive immune response by stimulation of T cells and B cells. Depending on the pattern of cytokine secretion, DCs are further capable of differentially polarising the T-cell response along the Th1/pro-inflammatory (interleukin (IL)-12) or Th2/immunosuppressive (IL-10) pathways. On account of evidence that DCs are the most effective antigen-presenting cell for stimulation of T-cell responses to a range of viruses, and considering their prime location at mucosal sites, DCs are considered to be the major players in initiation of the

primary antiviral immune response.<sup>9</sup> Similarly, they are central to the induction of vaccine responses, as they are the only antigen-presenting cell that can stimulate naive T cells to differentiate to 'experienced' memory T cells.

The impact of obesity on myeloid DC number and function,<sup>10–12</sup> and associated viral responses,<sup>13,14</sup> has been described in several reports to date. The aim of this study was to further examine the effect of obesity on human DC function and T-cell instruction.

## MATERIALS AND METHODS

Preparation of peripheral blood mononuclear cell and myeloid DCs

Peripheral blood mononuclear cells were isolated by density centrifugation. CD14<sup>+</sup> monocytes were magnetically isolated from peripheral blood mononuclear cells according to the protocol described by Miltenyi Biotech separation systems (Surrey, UK). Innate myeloid DCs were generated by culturing CD14<sup>+</sup> cells for 6 days in RPMI supplemented with 10% fetal bovine serum (Hyclone, Hampton, NH, USA),  $50 \text{ ng ml}^{-1}$  granulocyte-macrophage colony-stimulating factor and  $70 \text{ ng ml}^{-1}$  IL-4 (Immunotools, Friesoythe, Germany). Supplemented medium was replenished on day 3 and DCs were harvested on day 6.

## Flow cytometric analysis

Circulating DC enumeration was performed by staining peripheral blood mononuclear cells with lineage cocktail, CD11c and HLA-DR (BD Bioscience, Oxford, UK). Cultured DCs were analysed using a FACSCalibur flow cytometer and CellQuest Pro software (BD Bioscience). Cells were gated ('monogate') by their density and granularity. Results are expressed

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as a percentage of the monogate or the percentage/fold change in mean fluorescence intensity.

### Effect of obesity on DC function

DCs from either obese or lean donors were cultured in the absence or presence of  $10 \text{ ng ml}^{-1}$  lipopolysaccharide (LPS) or poly I:C for 24 h. DCs were stained for phenotypic analysis with mAbs specific for the surface molecules CD83 and CD86 (BD Bioscience) and relevant controls. Surface marker expression was expressed as fold increase over DCs cultured in medium alone. Cell supernatants were assessed by enzyme-linked immunosorbent assay and FlowCytomix for cytokine secretion. Cytokine levels are expressed as fold increase in production over the levels produced by DCs cultured in media alone.

### Measurement of cAMP response element-binding protein (CREB) phosphorylation

Monocyte-derived DCs generated from either lean or obese subjects ( $3 \times 10^6$ ) were cultured with or without LPS ( $100 \text{ ng ml}^{-1}$ ) for indicated times. Cell lysates were generated and were then resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane and probed for immunoreactivity using anti-phospho-CREB (Santa Cruz, Dallas, TX, USA), -CREB (Santa Cruz) and  $\beta$ -actin (Sigma, Wicklow, Ireland) specific antibodies. Immunoreactive bands were detected using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA) according to the manufacturer's instructions. Densitometry was assessed using ImageJ software (National Institute of Health, Bethesda, MD, USA).

### T-cell cytokine production assay

Immature DCs ( $2 \times 10^5/250 \mu\text{l}$ ) from obese and lean donors were cultured with or without LPS for 24 h. After 24 h, immature DCs were co-cultured with freshly isolated lean healthy CD3+ T cells at 10:1 ratio for 72 h. Supernatants were assessed for cytokine production by enzyme-linked immunosorbent assay.

## RESULTS

### Weight, body mass index and metabolic parameters of lean and obese cohorts

Twenty grade III obese subjects were recruited from a hospital-based weight management unit. In addition, 10 lean volunteers were studied as a parallel control group. St Vincent's University Hospital Ethics Committee approved this study. Written informed consent was obtained from every participant before the start of any research activities.

The clinical characteristics of the study sample are detailed in Table 1.

### Number and function of circulating DCs in obesity

Obese individuals had significantly less circulating innate DCs than lean controls (0.82% vs 2.53%,  $P < 0.0001$ ; Figures 1a and b).

Monocyte-derived DCs from lean but not obese subjects upregulated the expression of CD86 in response to LPS. Obese donors expressed significantly less CD83 than lean controls both basally and post stimulation with either LPS or poly I:C (Figures 1c and d). LPS or poly I:C stimulation of immature DCs from lean and obese subjects resulted in significantly increased levels of IL-10, however, obese DCs produced significantly higher levels of IL-10 than lean controls (Figure 1e). Obese DCs produced significantly less IL-12 in response to poly I:C but no significant difference was observed with LPS stimulation (Figures 1g and i). LPS stimulation of immature DCs from obese subjects resulted in significantly higher levels of phosphorylation of the CREB transcription factor (Figures 1j and k). T cells co-cultured with LPS-stimulated DCs from obese donors produced significantly higher levels of the Th2 cytokine IL-4 compared with lean controls (Figure 1l).

## DISCUSSION

Obesity is known to impair the innate immune system.<sup>15,16</sup> The impact of obesity on the cells of the adaptive immune system has also been reported.<sup>17</sup> DCs are the bridge between innate and adaptive immune responses. They are responsible for activation and polarisation of T cells, providing multiple signals that shape the adaptive immune response.<sup>18</sup> Here we report that DCs generated from obese donors are significantly altered in both cytokine production and T-cell-stimulating abilities when compared with lean controls.

Obese DCs expressed significantly lower levels of CD86, a molecule that provides the co-stimulation signal, which is required for full T-cell responses. Obese DC also expressed significantly lower levels of CD83 compared with lean DCs. CD83 is classically described as a DC maturation marker, however, more recently it has been implicated in T- and B-cell responses.<sup>19</sup> Several viruses influence CD83 expression, thereby inhibiting T-cell activation. Herpes simplex virus-1 and human cytomegalovirus target and deplete CD83 surface expression as an immune escape mechanism.<sup>20,21</sup> Reduced expression of CD83 on stimulated obese DCs may contribute to the increased susceptibility to viral infection and the defective response to vaccination against viruses seen in obese individuals.<sup>5-7</sup>

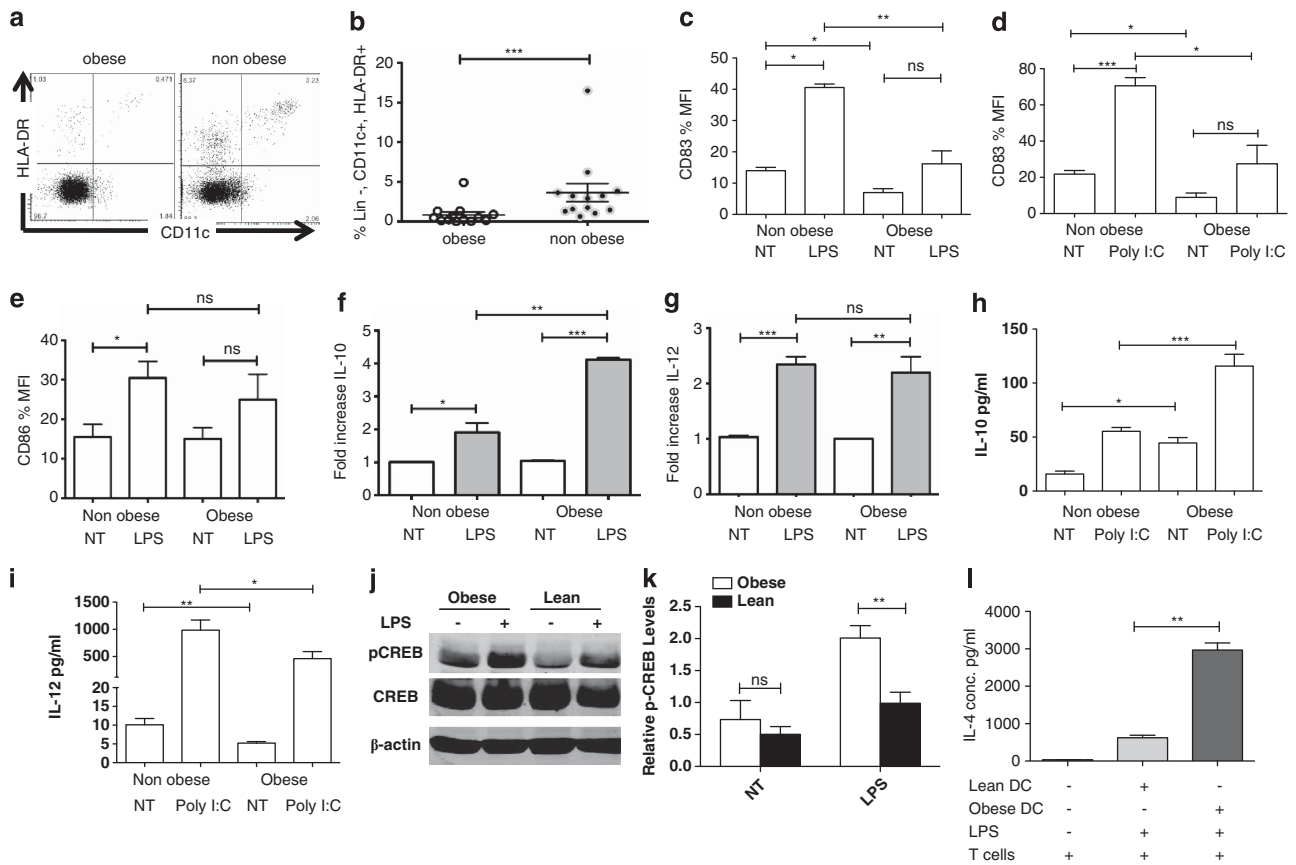
Impairment of DC number and functionality has previously been reported in murine studies. DCs from ob/ob mice showed dysregulated cytokine responses to antigenic stimulation when compared with lean controls.<sup>11</sup> Diet-induced obese mice showed altered and inefficient T-cell responses to influenza infection, attributed to upregulation of immunosuppressive cytokines from lung DCs.<sup>22</sup> This study reports similar findings in severe human obesity, demonstrating high levels of the immunosuppressive cytokine IL-10 in response to LPS stimulation of obese DCs, and increased phosphorylation of the immunosuppressive transcription factor CREB. Furthermore, we show a significant increase in the production of the Th2 cytokine IL-4 from T cells co-cultured with obese DCs. The immunosuppressive profile of cytokines produced by obese DCs, paired with the instruction of T cells to produce significantly higher levels of IL-4, supports our hypothesis that defective DCs may contribute to the increased risk of viral infections in obesity.

Weber *et al.*<sup>23</sup> reported that higher body mass index was the single best predictor of failure to develop detectable antibodies to hepatitis B vaccination. The antibody response to tetanus vaccination was shown to be significantly reduced in overweight children.<sup>24</sup> Higher body mass index was associated with a greater decline in antibody titres to influenza at 12 months post-vaccination.<sup>6</sup> However, little is known about the mechanism underlying the adverse impact of obesity on response to vaccination. The delicate balance of memory T-cell creation has been described using a 'Goldilocks model', where memory T-cell generation is best when the immune environment is 'just right'.<sup>25</sup>

**Table 1.** Clinical characteristics of the study sample

	Obese	Lean
Gender (M:F)	5:15	3:7
Age (years)	39.3 ± 11.8	33.3 ± 7.0
Weight (kg)	143.6 ± 26.6	76.7 ± 23.9
BMI ( $\text{kg m}^{-2}$ )	51.7 ± 7.3	24.4 ± 3.1
Fasting glucose ( $\text{mmol l}^{-1}$ )	5.2 ± 0.6	4.6 ± 0.3
HbA1c ( $\text{mmol mol}^{-1}$ )	41 ± 4	33 ± 1
Total cholesterol ( $\text{mmol l}^{-1}$ )	5.3 ± 1.1	4.2 ± 0.9
Triglyceride ( $\text{mmol l}^{-1}$ )	1.9 ± 1.2	1.0 ± 0.3
LDL-cholesterol ( $\text{mmol l}^{-1}$ )	3.5 ± 0.9	1.6 ± 1.0
HDL-cholesterol ( $\text{mmol l}^{-1}$ )	1.3 ± 0.2	0.4 ± 0.7

Abbreviations: BMI, body mass index; F, female; HDL, high-density lipoprotein; M, male; LDL, low-density lipoprotein.



**Figure 1.** Obesity impairs DC cytokine production and T-cell instruction. (a) Circulating DCs were identified by electronically gating on lineage 1 negative and CD11c<sup>+</sup>, human leukocyte antigen (HLA)-DR<sup>+</sup> cells as shown in representative dot plots from obese and non-obese subjects. (b) Scatter plot showing the mean DC number in obese and non-obese cohorts ( $n = 12$ ). (c, d) Bar graphs showing the mean fluorescence intensity expression of CD83 by non-obese and obese myeloid DCs either unstimulated or stimulated with  $10 \text{ ng ml}^{-1}$  of LPS or poly I:C for 24 h. (e) Bar graphs showing the mean fluorescence intensity expression of CD86 by non-obese and obese myeloid DCs either unstimulated or stimulated with  $10 \text{ ng ml}^{-1}$  of LPS for 24 h. (f, g) Bar graphs showing production of IL-10 and IL-12 by non-obese and obese DCs either unstimulated or stimulated with  $10 \text{ ng ml}^{-1}$  of LPS for 24 h. (h, i) Bar graphs showing production of IL-10 and IL-12 by non-obese and obese DCs either unstimulated or stimulated with  $10 \text{ ng ml}^{-1}$  of poly I:C for 24 h. (j) Western blot showing the induction of phosphorylation of CREB post-LPS stimulation in obese and non-obese DCs. (k) Densitometry bar graph showing relative fold increase in LPS-induced phosphorylation of CREB in obese and non-obese DCs. (l) Bar graph showing production of IL-4 by allogeneic peripheral T cells from non-obese healthy donors instructed by DCs generated from either obese or non-obese subjects stimulated with  $10 \text{ ng ml}^{-1}$  LPS for 24 h. Supernatant cytokines measured by enzyme-linked immunosorbent assay. \* $P = 0.05$ , \*\* $P = 0.01$ , \*\*\* $P = 0.001$ . MFI, mean fluorescence intensity; NS, not significant; NT, no treatment.

We demonstrate dysregulated cytokine production and T-cell stimulation, in the context of the chronic low-grade inflammatory state of obesity.

Adult and childhood obesity levels are increasing worldwide. The associated increased susceptibility to viral and bacterial infections is already impacting on the medical, economic and social burden of overweight and obesity. This study focused on DCs, demonstrating reduced DC numbers, and a diminished ability to elicit appropriate T-cell responses to a general stimulus in obese individuals. This critical defect in the immune response may contribute to the increased susceptibility to viral infection and the poor vaccination response to certain viruses seen in obesity.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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