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Uremia impairs blood dendritic cell function in hemodialysis patients

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Patients on hemodialysis have a general immunodeficiency involving both innate and adaptive responses. As the mechanisms contributing to this defect are uncertain, we sought to study the effects of uremia on circulating dendritic cells (DC) in hemodialysis patients. Immunomagnetic beads were used to isolate myeloid and plasmacytoid DCs from healthy donors. Immune-related functions were determined in these cells cultured in either a complete media containing ABO-compatible serum or media containing sera from uremic patients. The myeloid cells were analyzed for costimulatory molecule expression and allo-stimulatory capability following lipopolysaccharide stimulation. The production of interferon-α following herpes-simplex virus stimulation by the plasmacytoid cells was also measured. Myeloid DCs incubated with uremic sera demonstrated impaired maturation and decreased allo-stimulatory capacity. Similarly, herpes virus-stimulated plasmacytoid DCs incubated with uremic sera produced significantly less interferon-α compared with cells incubated in the complete media. Both small and large molecule uremic toxins inhibited DC functions *in vitro*. Use of more efficient dialysis to improve small molecule clearance reversed the inhibition of uremic sera on myeloid but not plasmacytoid DC function. We have shown that the immunodeficiency of hemodialysis patients is due to dialyzable uremic toxins.

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Infectious complications are a common cause of morbidity and mortality in end-stage renal disease (ESRD) patients maintained on renal replacement therapies (e.g. hemodialysis (HD)).[1,2] In addition, ESRD patients often have a poor response to vaccinations (e.g. hepatitis B) achieving low therapeutic antibody titres,[3] and have a higher risk of malignancies.[4] These observations suggest an underlying generalized defect in both innate and adaptive immunity, but the underlying mechanisms remain unclear.[5]

Dendritic cells (DC) are unique professional antigen-presenting cells that coordinate both innate and adaptive arms of the immune system.[6] DC are critical in stimulating effector cells (including naïve T cells and natural killer cells) in response to captured foreign antigens.[7] There are at least two distinct subsets of circulating DC precursors derived from $CD34^+$ bone-marrow hematopoietic progenitor cells in the peripheral blood (PB). These include precursor myeloid and precursor plasmacytoid DC,[8–10] which can be easily identified and isolated by flow cytometric cell sorting or via immunomagnetic bead separation technique.[11,12] Precursor myeloid DC (MDC) are derived from myeloid precursors, express CD11c and blood dendritic cell antigen (BDCA)-1 (CD1c).[12] These cells are immunosurveillant cells and drive a potent T-helper (Th) 1-type polarized immune response to lipopolysaccharide (LPS).[9] Plasmacytoid DC (PDC) are a recently described DC subset that express cell-surface markers CD123, BDCA-2 and 4.[12] PDC are critical in antiviral and possibly antitumour immunity by producing large amounts of type I-interferon (IFN).[13–15] The effect of renal failure on DC function has not been extensively investigated.

In a previous study, we established that immunomagnetic bead isolated uremic MDC and PDC from HD patients were functionally impaired *ex vivo* with reduced cell-surface costimulatory molecule expression and IFN-α production following appropriate stimulation.[16] We hypothesized that uremic toxins accumulated at high concentrations in the sera of HD patients inhibit blood DC functions *in vivo*. In this study, we assessed the impact of uremic sera of HD patients on MDC and PDC functions *in vitro*. We demonstrate that uremic toxins of all molecular weights (MWs) contained within the sera of HD patients inhibit DC function, and that these effects are potentially reversable by more efficient dialysis. Our findings provide a rationale for utilizing HD

regimens to improve clearance of uremic toxins and enhance overall immune function in HD patients.

RESULTS

HD patients have higher serum levels of IL-12p70

Serum interleukin (IL)-12p70 levels were significantly higher in HD patients ($n=12$, 43.1 ± 11.0 pg/ml) compared with healthy controls/human AB serum ($n=14$, below level of detection; Mann–Whitney *U*-test $P<0.001$).

UM inhibits normal BDCA-1$^+$ MDC functions

To determine the effect of uremic serum on BDCA-1$^+$ MDC function, we studied *normal* BDCA-1$^+$ MDC cultured in uremic medium (UM). UM blocked costimulatory (CD40, CD80, and CD86) and maturation (CD83) markers on LPS-stimulated *normal* BDCA-1$^+$ MDC (sera of >10 separate HD patients; Figure 1a). These findings translated to a significantly reduced allo-stimulatory capacity in mixed lymphocyte reaction (sera of five separate HD patients, Mann–Whitney *U*-test $P<0.001$; Figure 1b).

Compared with complete medium (CM)-cultured BDCA-1$^+$ MDC, UM-cultured cells exhibited enhanced CD95 expression (sera of >10 separate HD patients, mean fluorescent intensity (MFI) 5.3 ± 3.8 compared with 10.6 ± 6.8, respectively, $P=$ not statistically significant (NS)), but similar proportion of annexin and/or propidium iodide (PI)-positive cells (sera of three separate HD patients, $49.8\pm13.2\%$ compared with $57.2\pm10.4\%$ of total cells, respectively, $P=$NS). LPS-stimulated BDCA-1$^+$ MDC cultured in UM compared with CM produced a greater amount of IL-12p70 (sera of >10 separate HD patients, 58.9 ± 10.4 pg/ml compared with 25.2 ± 5.2 pg/ml, Mann–Whitney *U*-test $P<0.01$). The amount of IL-12p70 present in CM and UM was below level of detection and 9.2 ± 4.5 pg/ml,

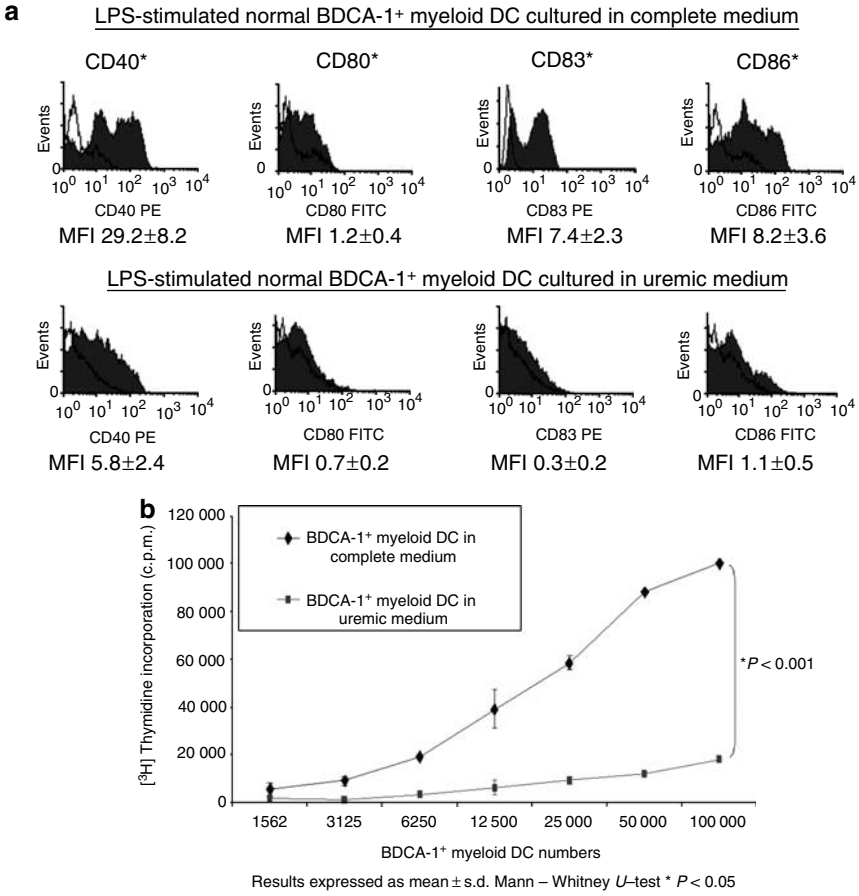

Figure 1 | Phenotypic expression and functional analysis of BDCA1+ MDC. (**a**) Uremic medium inhibits the expression of costimulatory molecule on BDCA-1+ MDC. Cell-surface expression of costimulatory molecules on LPS-stimulated (10 ng/ml) *normal* BDCA-1$^+$ MDC of healthy blood donors cultured in either CM or UM for 24 h. Flow cytometric analysis revealed reduced cell surface expression of costimulatory molecules (CD40, CD80, and CD86) and CD83 on LPS-stimulated BDCA-1$^+$ MDC cultured in UM compared to LPS-stimulated BDCA-1$^+$ MDC cultured in CM (closed profiles). Open black profiles denote isotype controls. Representative histograms shown. MFI (mean$\pm$s.d.) below histograms representative of results of >10 experiments involving the sera of >10 separate HD patients (Mann–Whitney *U*-test $*P<0.01$). (**b**) Allogeneic T-cell proliferation in a BDCA-1$^+$ MDC/T cells two-way mixed lymphocyte reaction. LPS-stimulated (10 ng/ml) *normal* BDCA-1$^+$ MDC of healthy blood donors cultured in CM for 24 h induced greater allogeneic T-cell proliferation (measured as tritiated thymidine incorporated cells) compared with *normal* BDCA-1$^+$ MDC cultured in UM for 24 h (Mann–Whitney *U*-test $P<0.001$). Result representative of five separate experiments involving the sera of five separate HD patients.