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The cellular immune response to Cytomegalovirus is associated with expansion of CCR2 expressing monocytes that in turn are linked to increased proteinuria in patients with ANCA associated vasculitis

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Background

We have previously shown that subclinical reactivation of cytomegalovirus (CMV) drives the expansion of CMV specific T-cells such as CD4+CD28null T-cells in patients with ANCA associated vasculitis (AAV). Expansion of CD4+CD28null T cells in AAV is in turn linked to decreased renal function, increased arterial stiffness and increased mortality.

Recent evidence in diabetic kidney disease suggests that expansion of pro-inflammatory monocyte subsets, such as CCR2 expressing monocytes, is associated with arterial inflammation, as well as proteinuria and renal injury. CCR2 blockade ameliorates inflammation and renal injury in diabetic mice and has shown promise in reducing proteinuria in phase 2 clinical trials in diabetic kidney disease.

We hypothesized that subclinical reactivation of CMV may be associated with differential expansion of monocyte subsets and CCR2 expression in patients with AAV, and that this may contribute to arterial stiffness and renal injury.

Methods

Cryopreserved peripheral blood mononuclear cell samples from 32 CMV seropositive AAV patients with stable disease that took part in a previous study were stained with monoclonal antibodies to assess expression of CD3, CD56, CD14, CD16, CCR2 and CX3CR1 in order to enumerate classical (CD14++CD16-), non-classical (CD14+CD16++) and intermediate (CD14++CD16+) monocytes, as well as CCR2 expressing monocytes.

Paired data from the same patients and time-point were available for CMV-specific T-cell percentage (determined by CD4 T-cell interferon-γ secretion following overnight CMV lysate stimulation), CD4+CD28null T-cell percentage (a marker of recent subclinical CMV reactivation), plasma concentration of monocyte chemoattractant protein 1 (MCP-1), carotid to femoral pulse wave velocity (PWV; arterial stiffness), peripheral pulse pressure, serum creatinine and eGFR, and urinary albumin creatinine ratio (ACR). Flow cytometry data were analysed using DIVA Version 7 software. Analyses were conducted using SPSS Version 21.

Results

The size of the CD4+CD28null T-cell expansion was correlated with CCR2 expressing monocytes (rho=0.390, p=0.030). CMV-specific T-cell percentage also correlated with CCR2 expression in monocytes (rho=0.395,

p=0.028). There was no significant association seen between CD4+CD28nul T-cells or CMV-specific T-cells and classical, intermediate or non-classical monocyte subsets. The size of the CCR2 expressing monocyte compartment was positively correlated with peripheral pulse pressure (rho=0.377, p=0.040) but not pulse wave velocity.

CCR2 expressing monocytes were positively correlated with ACR as a marker of proteinuria (rho=0.432, p=0.015). CCR2 expressing monocytes also positively correlated with MCP-1 in plasma, the chemokine associated with targeting monocytes to inflammatory sites (rho=0.457, p=0.010). MCP-1 in turn was positively correlated with serum creatinine (rho=0.523, p=0.003) and proteinuria (rho=0.443, p=0.013) and negatively correlated with eGFR (rho = -0.458, p=0.010).

Discussion

Our results suggest that subclinical reactivation in AAV may be associated with expansion of CCR2 expressing monocytes. We observed that expansions of CCR2 expressing monocytes were associated with higher levels of proteinuria.

Our data, although preliminary, suggest that subclinical reactivation of CMV may contribute to proteinuria and renal injury in AAV patients with renal involvement via the expansion of pro-inflammatory CCR2 expressing monocytes.