Targeting of Therapeutic Chimeric Antigen Receptor Macrophages (CARM) : Temporal Changes in the Renal Cytokine Profile in Response to AA Amyloidosis Induces Macrophage Infiltration

Manasi Balachandran, Trevor Hancock, Tina Richey, Sallie Macy, Craig Wooliver, and Jonathan Wall

University of Tennessee Graduate School of Medicine, Knoxville, TN, USA

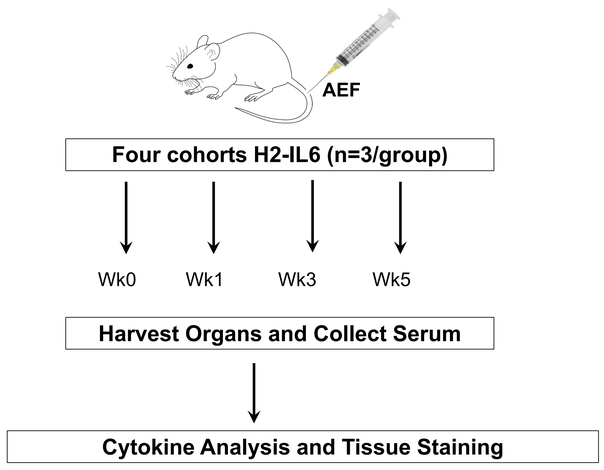
Introduction

The deposition of systemic amyloid is a complex process which, in contrast to amyloid in the brain, does not result in an inflammatory response nor the recruitment of phagocytic macrophages and monocytes capable of clearing the amyloid. However, studies of the inflammatory microenvironment in patients with amyloid are now underway which may lead to novel therapeutic opportunities.

In parallel, we have generated chimeric antigen receptor-macrophages (CARM) that express the pan-amyloid-reactive peptide, p5, to facilitate amyloid recognition, binding and clearance. Cell-based therapies do not have targeting capabilities but accumulate in sites of inflammation. Amyloid is typically a non-inflammatory pathology; however, we have observed trafficking of macrophages to the kidney in a murine model of AA amyloidosis. Here, we describe the temporal changes in cytokine levels during the development of murine AA amyloidosis, that contribute to this phenomenon.

Methods

Four cohorts of H2-IL6 mice were administered amyloid enhancing factor (AEF) intravenously (*n*=3/group). At different time points T0 (before AEF injection), T1 (1-week post-AEF), T2 (3 weeks post-AEF) and T3 (5 weeks post-AEF), serum samples and organs (kidney, liver, spleen, pancreas, and heart) were harvested from each mouse. Serum and tissue-derived cytokine levels were quantified using the Legendplex Mouse Anti-Virus Response Kit™ and analyzed by flow cytometry. Tissues were fixed in formalin and sections were stained with Congo red (for amyloid), H&E, Iba-1 and CD68 (for macrophage identification and activation).



Results:

In our previous studies with CMFDA-labeled CARMs, we observed that when injected IV, these cells specifically migrated to the kidneys in H2-IL6 AA mice and not any other organ (unpublished data, Fig 1A & 1B). Correspondingly, in the kidneys, we observed a significant increase in pro-inflammatory cytokines [CXCL1 (p<0.0001), CXCL10 (p<0.0001), TNF-α (p<0.0010), CCL2 (p<0.0033)] between wk 1 and 3 post-AEF, concurrent with the progressive deposition of amyloid (Fig. 2A-B & Fig 3). This also coincided with an increase in Iba-1-positive macrophages in the kidney over the initial 3 weeks post-AEF (Fig. 2C-D). At wk 5 post-AEF renal cytokine levels returned to normal levels. Interestingly, no significant changes in organ-related cytokine production were seen as amyloid developed in the liver, pancreas, or spleen of the mice.

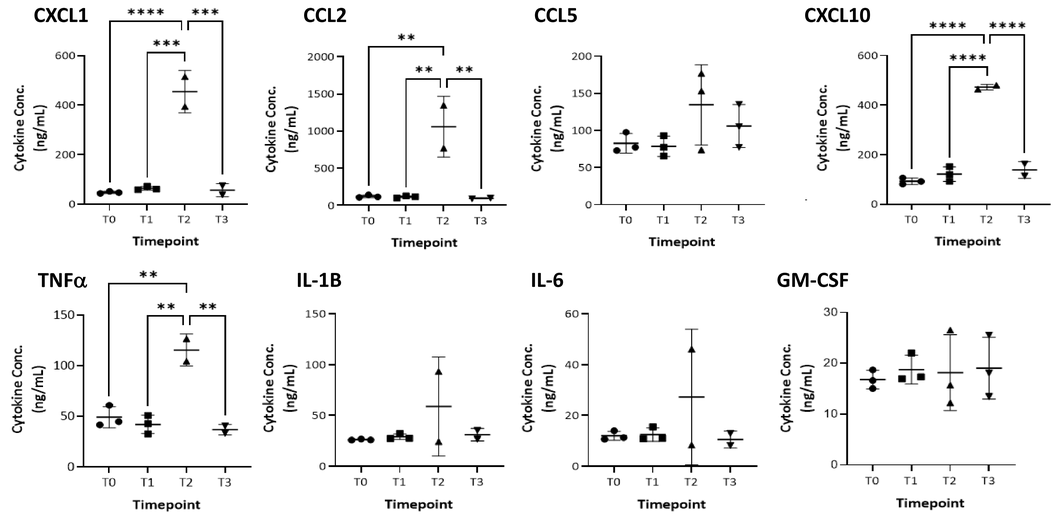


Fig. 3: Temporal changes in cytokine expression in the kidneys of AA mice (*n*=3) at wk0 (T0), wk1 (T1), wk3 (T2) and wk5 (T3) post-AEF. A significant increase in concentrations of CXCL1 (p<0.0001), CXCL10 (p<0.0001), TNF-α (p<0.0010), CCL2 (p<0.0033) were observed at T2 coincident with amyloid deposition and macrophage infiltration. All cytokines returned to normal levels at T3 as the amyloid deposition progressed to high levels.

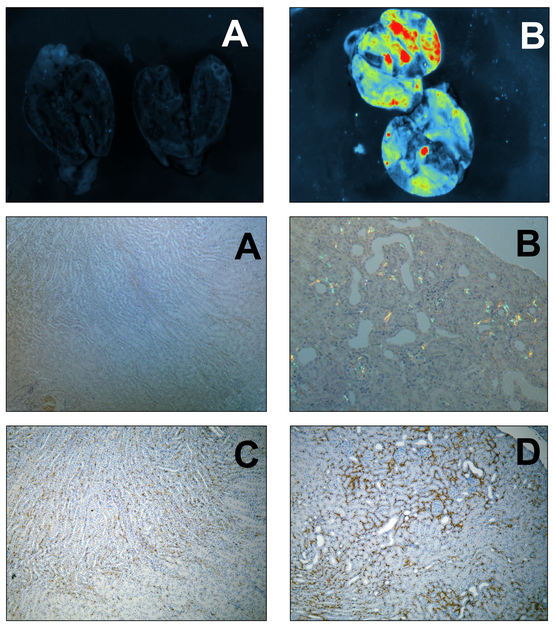


Fig. 1A and 1B: CAR-M cells injected into AA mice migrate specifically to the kidneys but not any other organ. No kidney trafficking is seen in the WT mice. CMFDA-labeled CAR-macrophages ($1x10^6$) were injected IV into WT (A) and H2-IL6 AA (B) mice showed kidney-specific trafficking of cells, in the AA mice only, at 2 days post injection.

Fig. 2A and 2B: Congo Red staining of mouse kidney at week 0 prior to AEF injection (A) and 3-weeks post-AEF injection (B, enlarged) showing an accumulation of interstitial and glomerular AA amyloid and prominent tubular dilation. Regions with high amyloid also showed a greater local accumulation of macrophages.

Fig. 2C and 2D: Iba-1 staining of mouse kidney at week 0 prior to AEF injection (C) with dispersed macrophages in the renal medulla and 3-weeks post-AEF injection (D) showing dense focal macrophages in the renal cortex coinciding with the increase in amyloid deposition and macrophage-induced cytokine levels (see Fig. 3).

Conclusion:

Renal amyloidosis in the transgenic AA mice resulted in temporal changes in pro-inflammatory cytokine expression leading to recruitment of monocytes. No such response was seen in the other amyloid laden organs in the mouse. If a pro-inflammatory environs similarly occurs in patients with renal amyloidosis, amyloid-reactive CARM may afford a novel therapeutic intervention for amyloid clearance. This preliminary study enhances our understanding of the organ-specific and temporal production of inflammatory cytokines during amyloid progression and how changes in amyloid impact the inflammatory state of an organ opening the door for increased opportunities for therapeutic intervention .

Disclosure:

JSW is interim CSO, and a founding shareholder in Attralus. TR is a founding shareholder in Attralus. Attralus has licensed technology related to pan amyloid reactive peptides and related reagents from the University of Tennessee Research Foundation. JSW receives research funding from Attralus and the NIH. MB and JSW have patent rights in peptide-based CARM constructs.