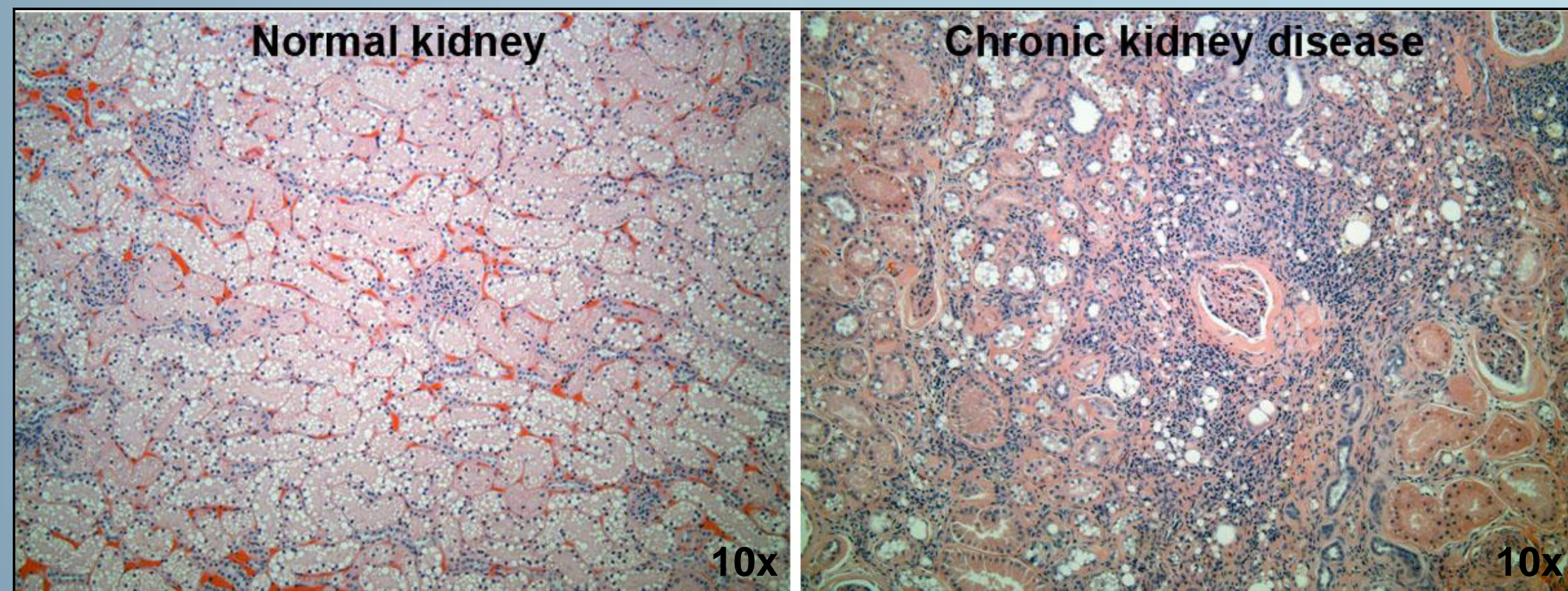


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## Introduction

- Chronic kidney disease (CKD) is a common disease of older cats, and remains a leading cause of morbidity and mortality among veterinary patients.
- Current treatment options are often limited to supportive therapy.
- The precise etiology of CKD is unknown, but histology shows a mononuclear infiltrate characteristic of chronic inflammation.



- Adipose-derived mesenchymal stem cells (aMSCs) can be grown *in vitro*, identified by their characteristic morphology, plastic-adherence in culture, and ability to differentiate into multiple lineages.
- The immunomodulatory effects of stem cell therapy have been demonstrated in a variety of *in vitro* and *in vivo* models.
- In a study by Semedo *et al.*, renal function improved after repeated intravenous (IV) mesenchymal stem cell administration in rats with induced CKD.

## Significance of Study

Although clinical trials using stem cell therapy have begun in both veterinary and human patients, the mechanism of action within the kidney is unknown. The purpose of this study is to help elucidate the paracrine anti-inflammatory effects of mesenchymal stem cells as a treatment for CKD.

### Hypotheses:

- Cats with chronic kidney disease have increased concentrations of inflammatory markers in urine or serum.
- Adipose-derived mesenchymal stem cells suppress the release of inflammatory mediators.

## Materials & Methods

### Isolation and Culture of Feline Adipose-derived Mesenchymal Stem Cells

- Adipose tissue biopsies were harvested from the inguinal region of specific-pathogen free cats in survival surgeries.
- Adipose tissue samples were collagenase-digested, and the resultant stromal vascular fraction was plated in plastic tissue culture flasks.
- Mesenchymal stem cells were selected by their adhesive properties, and passaged several times to increase cell numbers.

### T cell Proliferation and Cytokine Release Assay

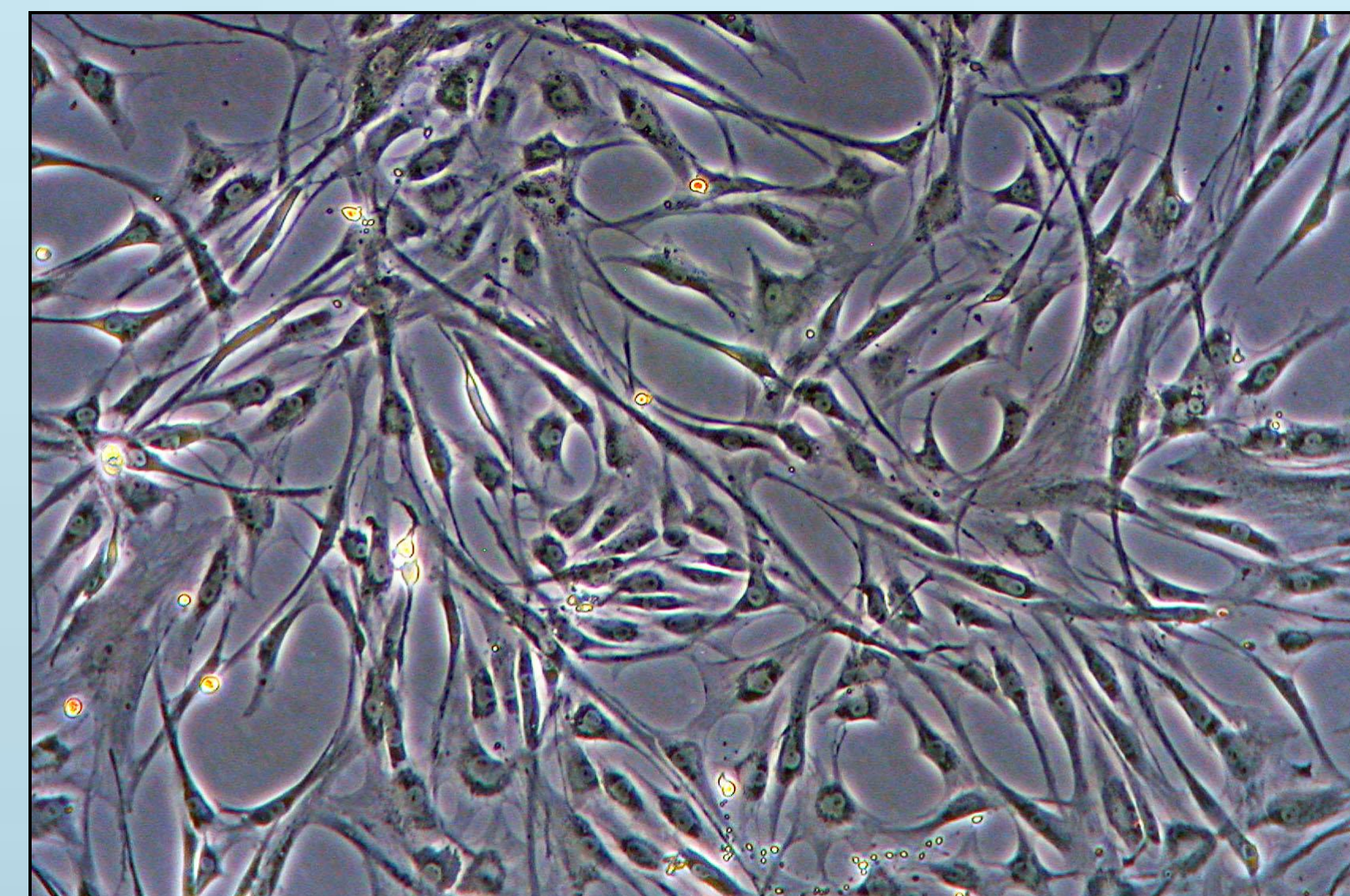
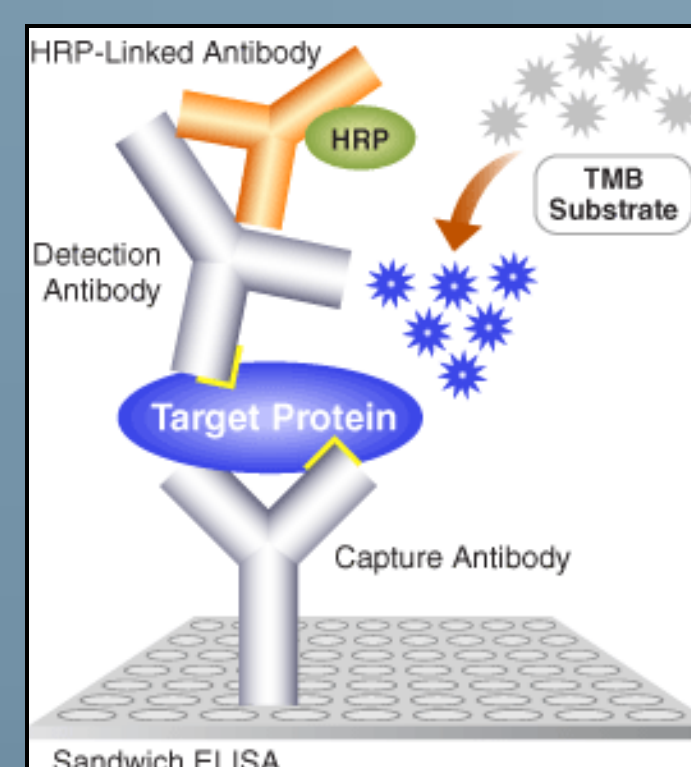
- Murine assay:** Peripheral blood mononuclear cells (PBMCs) were isolated from ICR spleens, stained with CFSE, and co-cultured with ICR aMSCs.

**Feline assay:** PBMCs were isolated from heparinized feline blood using lymphocyte separation medium and co-cultured with feline aMSCs.

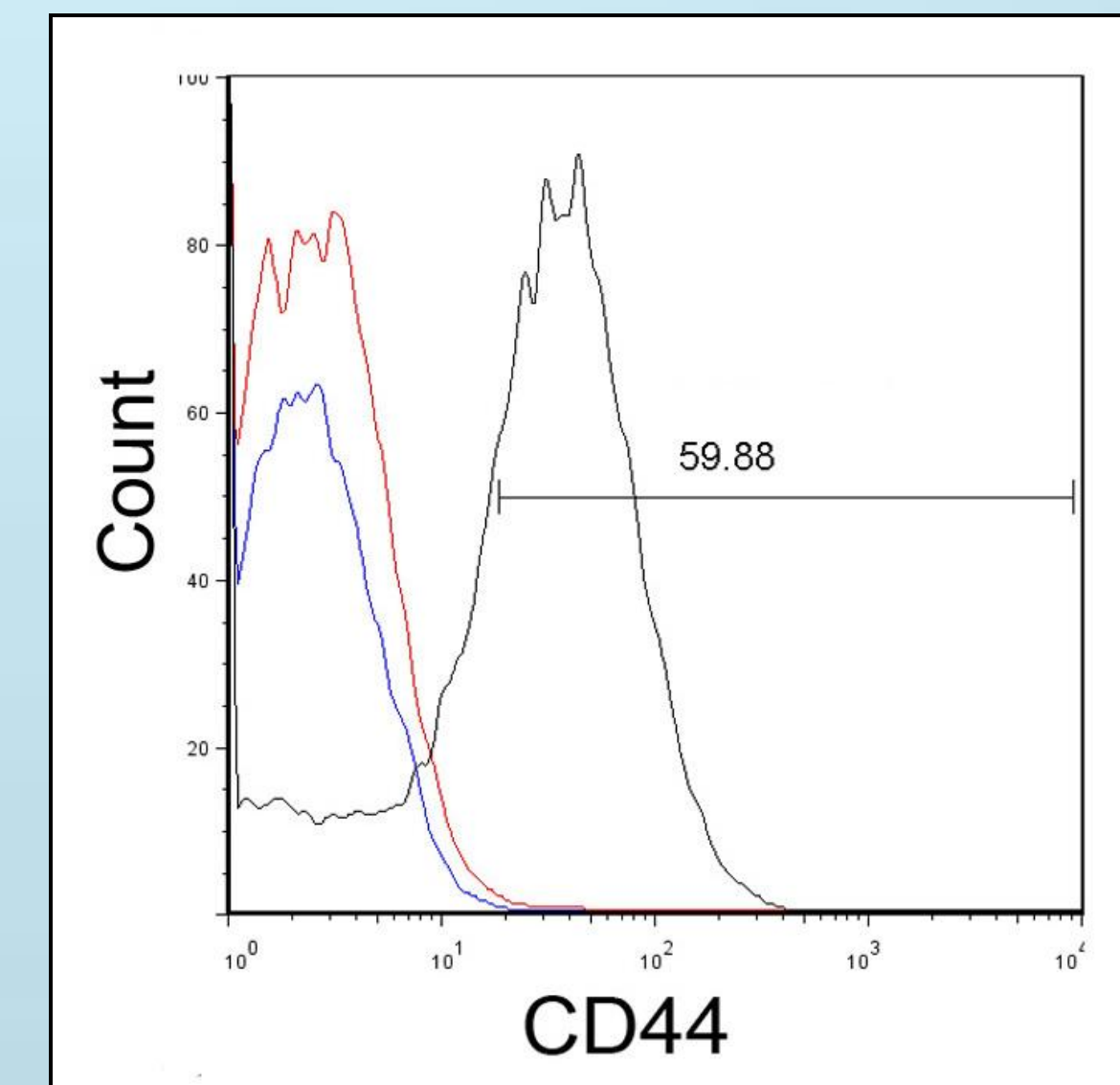
- Concanavalin A (conA, 5µg/ml) was added to the media to stimulate T cells.
- Supernatants were collected for ELISA analysis at 48hrs, and cells were collected for flow cytometry after 68 hrs.

### Evaluation of Inflammatory Markers in Feline CKD

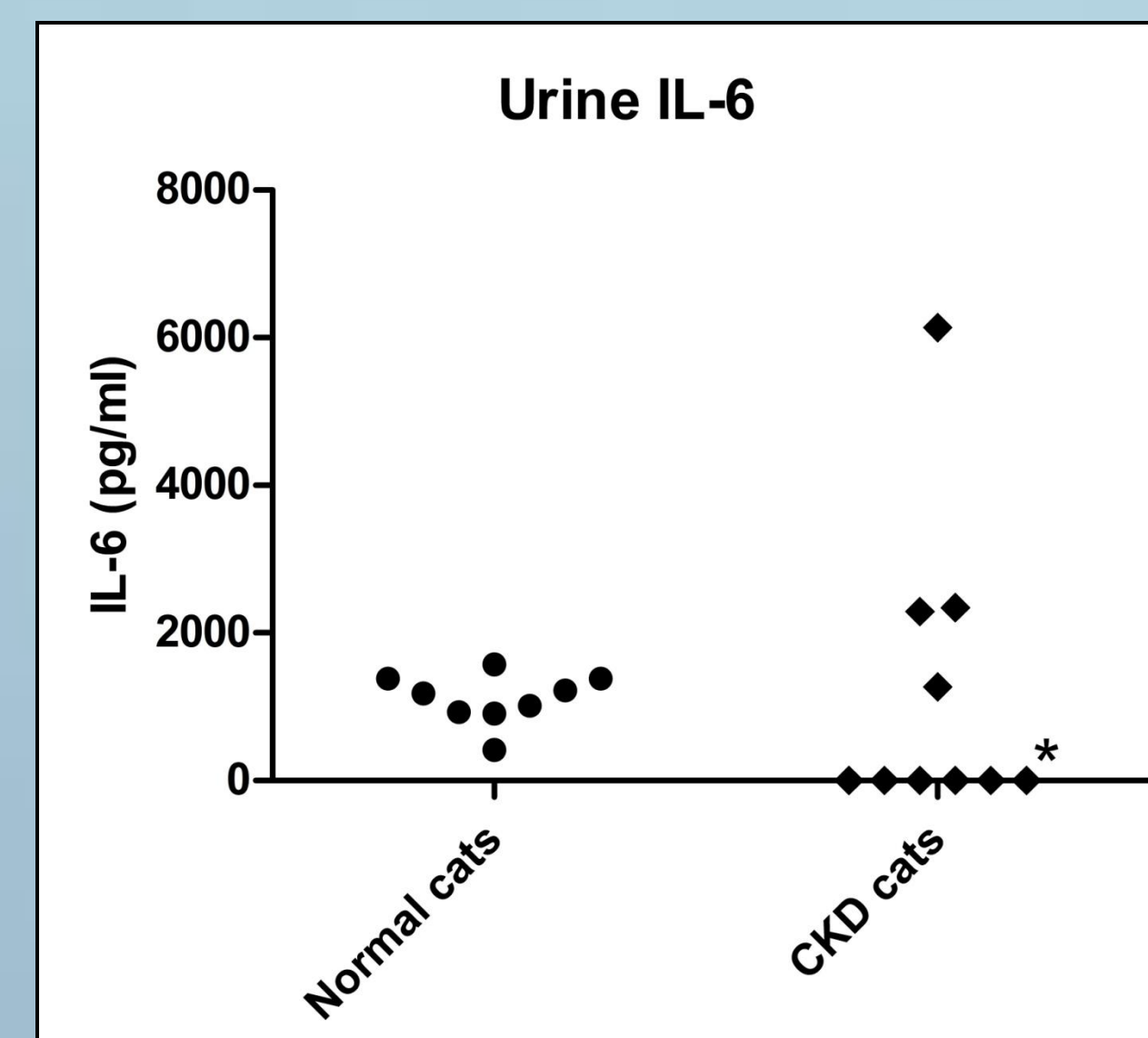
Serum and urine samples from normal cats and CKD cats were evaluated using optimized ELISA assays.



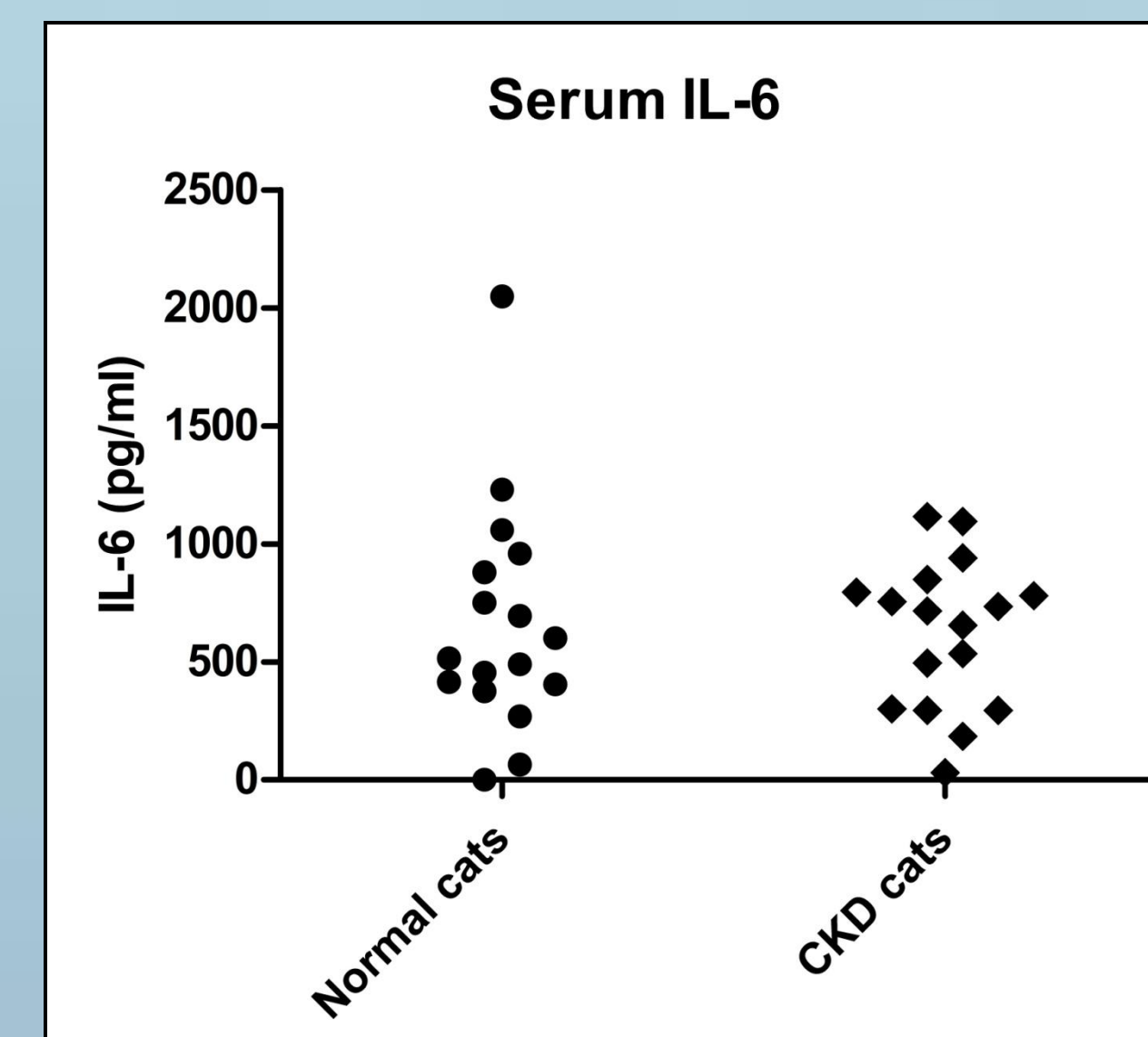
**Figure 1:** Feline adipose-derived aMSCs show characteristic fibroblast-type morphology and can undergo tri-lineage differentiation (data not shown).



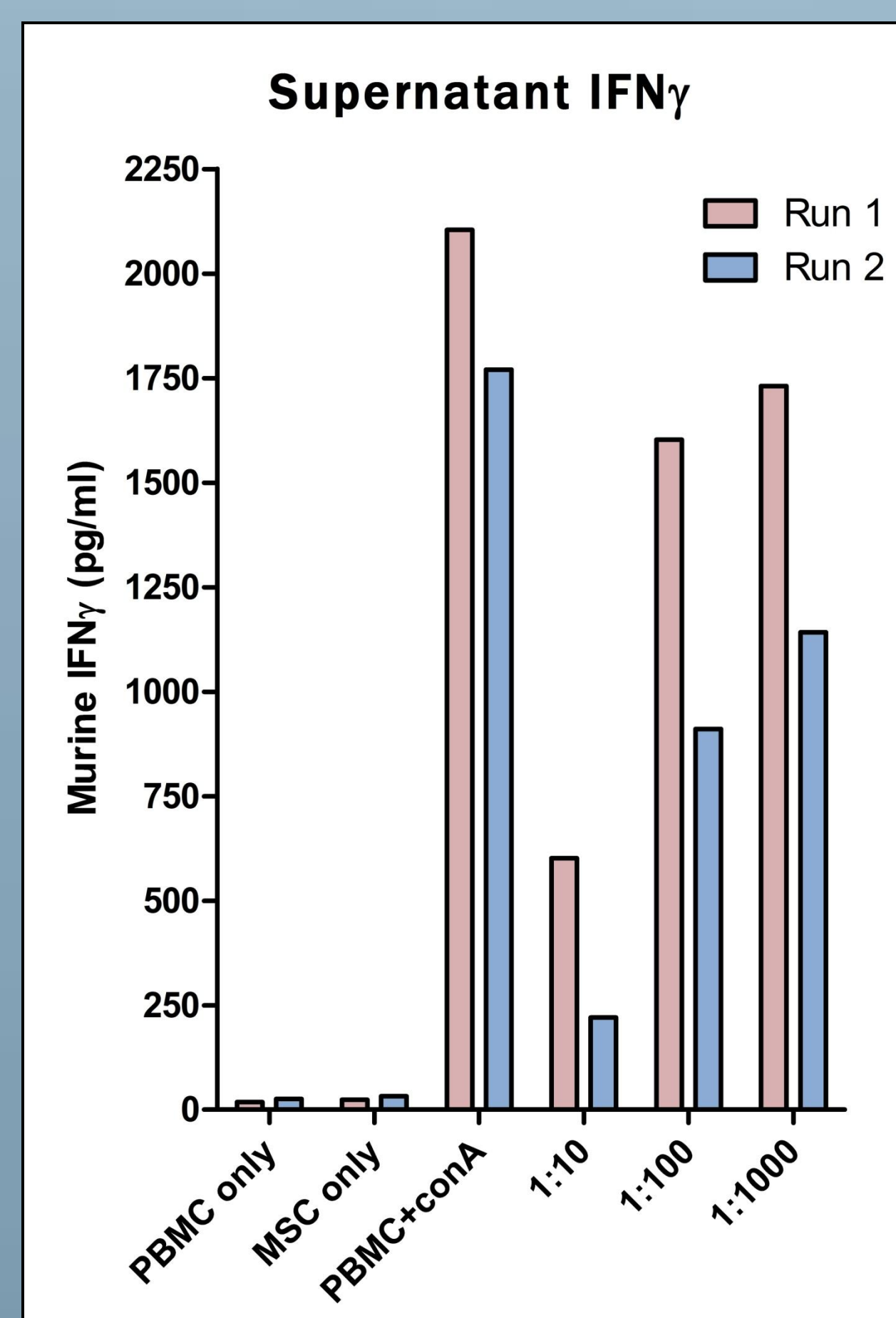
**Figure 2:** Cultured feline aMSCs are CD44+ (also CD105+, CD90+, MHC II-, and CD4-; data not shown), consistent with characterization of MSCs.



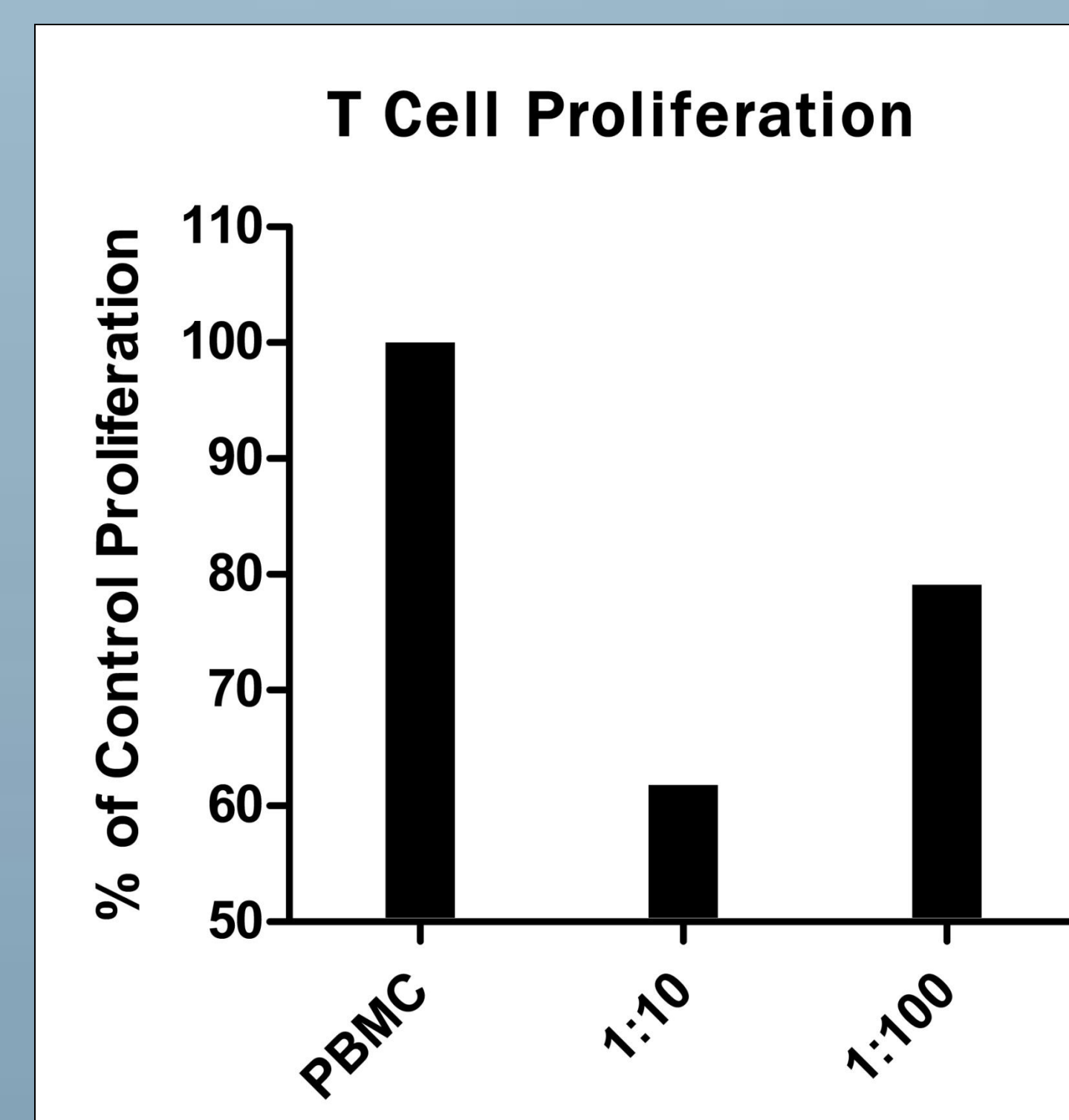
**Figure 4:** Preliminary results suggest that urine concentrations of IL-6 may be higher in cats with CKD, when corrected for urine dilution factors. \*Levels of IL-6 were below detection in some CKD samples.



**Figure 5:** Serum concentrations of IL-6 do not differ significantly between healthy and CKD cats.

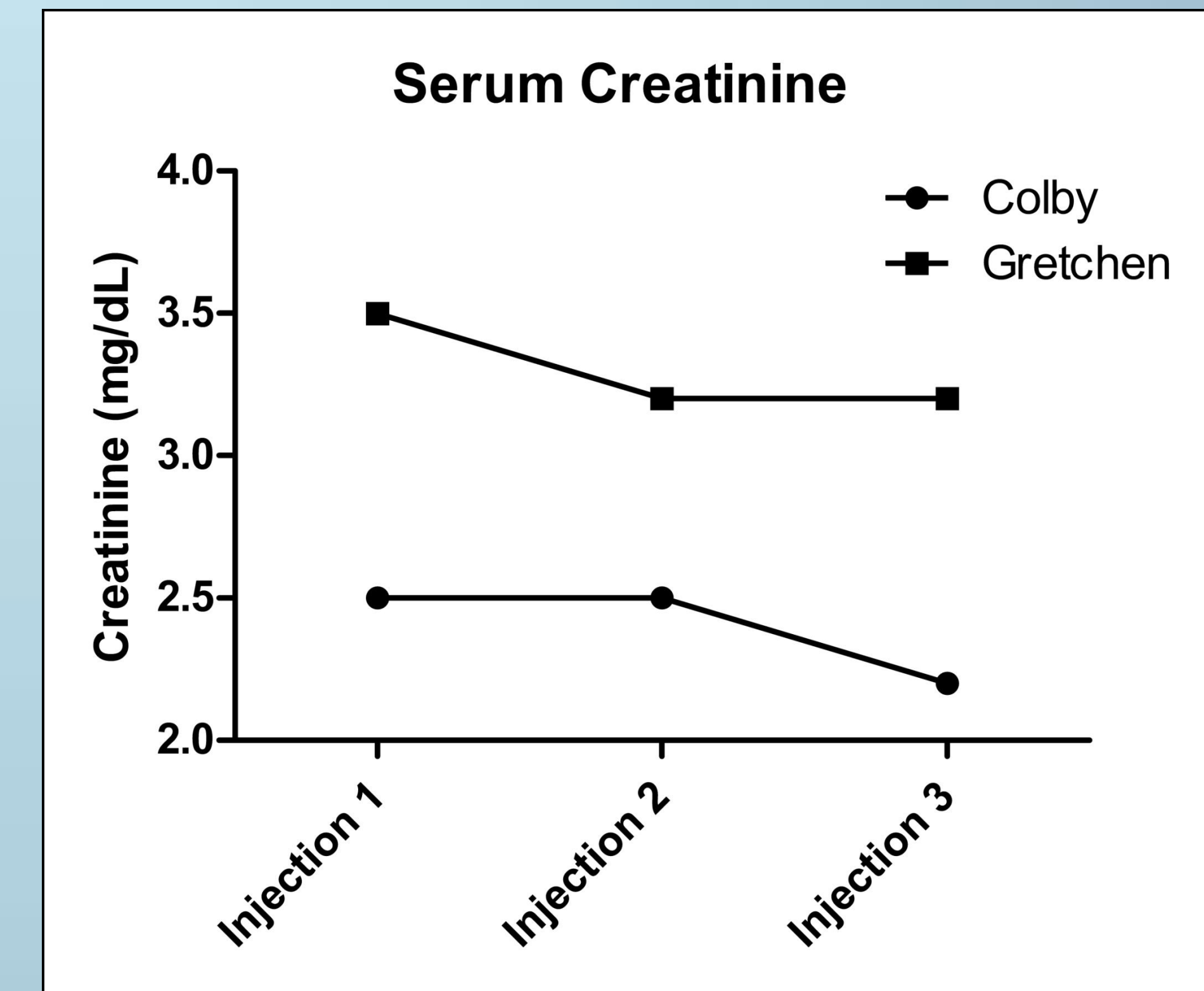


**Figure 6:** Murine aMSCs cause a dose-dependent decrease in IFN $\gamma$  secretion by conA activated T cells. Co-culture concentrations are shown as aMSC:PBMC.



**Figure 7:** Murine aMSCs suppress conA-induced T cell proliferation in a dose-dependent manner. Results shown as percentage of PBMC proliferation with conA alone.

## Results



**Figure 3:** Serum creatinine concentrations decrease in CKD cats treated intravenously with repeated q2week allogeneic aMSC injections.

## Discussion

- Feline aMSCs can be grown *in vitro*, using techniques established for harvest of murine and human aMSCs.
- Early data indicate that aMSCs represent a promising treatment for CKD-affected cats as repeated IV injection of allogeneic aMSCs resulted in decreased serum creatinine concentrations and increased body weight in some CKD cats.
- CKD cats have higher concentrations of the pro-inflammatory cytokine IL-6 in urine, suggestive of inflammation in the urinary tract. This is consistent with histopathologic findings of chronic renal inflammation in CKD patients. Serum concentrations of IL-6 are not significantly elevated in feline CKD patients.
- Murine aMSCs can alter inflammation by suppressing T cell proliferation and secretion of the pro-inflammatory cytokine IFN $\gamma$  in a dose-dependent manner.
- CKD in cats may be associated with increased pro-inflammatory cytokine concentrations. Improvement seen in aMSC-treated CKD cats may be due to the observed ability of aMSCs to suppress pro-inflammatory cytokine release.

## Future Directions

- Evaluate clinical samples for additional markers of inflammation, such as  $\alpha$ 1-acid glycoprotein, an acute phase protein.
- Perform additional cytokine analysis of feline and murine co-culture supernatants, including IL-8 and VEGF.
- Perform Inhibitory and transwell assays to further investigate the mechanisms of aMSC action *in vitro*.
- Continue enrollment of eligible CKD cats in allogeneic aMSC IV study.



## Acknowledgements

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