**DEFINING THE ANTI-INFLAMMATORY ACTIVITY OF M-T7,**

**A MYXOMAVIRAL CHEMOKINE MODULATOR THROUGH MUTAGENESIS**

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Viruses have co-evolved over millions of years with their hosts, creating a sophisticated yet potent class of anti-inflammatory and immune attenuating proteins. M-T7, a myxoma virus protein, is a chemokine modulating protein that inhibits chemotaxis by blocking chemokine binding to tissue glycosaminoglycans (GAGs). M-T7 prolongs allograft survival in rodent models. To investigate the mechanism of M-T7 anti-inflammatory activity, we have examined M-T7 and point mutants in vitro by cell migration and membrane fluidity and in vivo using a conditional NDST-/- (N-deacetylase/N-sulfotransferase responsible for sulfation of heparin sulfate) mouse model of aortic transplant and by balloon angioplasty. Point mutants of M-T7 were generated; residues were chosen for potential disruption of tertiary structure or interaction with chemokines. M-T7 and mutants F137D, R171E, and E209I inhibited RANTES mediated cell migration (P<0.001), but only M-T7 altered membrane fluidity of RANTES activated THP-1 monocytes. When THP-1 monocytes are activated by phorbol-12-myristate-13-acetate, M-T7 and F137D (P<0.029, P<0.028) altered membrane fluidity, but not R171E and E209I (P<0.016, P<0.005). In aortic allografts, M-T7 reduced plaque growth in C57BL/6 to BALB/c allografts (P<0.030) but not in NDST-/- to BALB/c (P<0.933). Further analysis of M-T7 mutants were performed in an ApoEnull balloon angioplasty model. R171E markedly increased plaque size (0.2770±0.125mm2) when compared to M-T7 while F137D and E209I reduced plaque, but less effectively than M-T7.

Conclusions:

1) M-T7 has significant anti-atherogenic activity in mouse allograft and balloon angioplasty models.

2) In vivo activity was only predicted in part by the in vitro activity of the M-T7 point mutants.

3) M-T7 has both chemokine dependent and independent anti-inflammatory activity.