IL1RAP Blockade Reduces Atherosclerosis and Limits Plaque Inflammation

MEGAN MULHOLLAND[1], GABRIEL JAKOBSSON[1], IRENA LJUNGCRANTZ[1], ANDRIETTA GRENTZMANN[1], CAITRIONA GRÖNBERG[2], SARA RATTIK[2], ALEXANDRU SCHIOPU[1], HARRY BJÖRKBACKA[1], DANIEL ENGELBERTSEN[1]

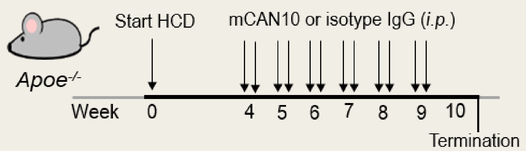
[1]DEPARTMENT OF CLINICAL SCIENCE, MALMÖ, LUND UNIVERSITY, SWEDEN; [2]CANTARGIA AB, LUND, SWEDEN. CONTACT: M. Mulholland: megan.mulholland@med.lu.se; D. Engelbertsen: daniel.engelbertsen@med.lu.se

Background

The interleukin-1 receptor accessory protein (IL1RAP) is required for signalling by IL-1α/β, IL-33, and IL-36α/β/γ. Both IL-1α and IL-1β have been shown to promote atherosclerotic plaque progression and inflammation, while the roles of IL-33 and IL-36 is still under investigation. An anti-IL1RAP antibody, CAN10, is currently in late-stage preclinical development for treatment of inflammatory and fibrotic diseases. Here we investigate the effects of a novel blocking non-depleting anti-mouse IL1RAP antibody on plaque burden and inflammation.

Methods

Apoe$^{-/-}$ mice were fed a high-cholesterol diet (HCD) and treated with biweekly *i.p.* injections of either anti-IL1RAP antibody mIgG2a-LALA-PG (mCAN10, provided by Cantargia AB) or isotype mIgG2a-LALA-PG (n=14/group) for six weeks starting at week 4 of HCD (see study outline below). Mice were terminated after a total of 10 weeks HCD and tissues were analysed by flow cytometry and histology.



Results

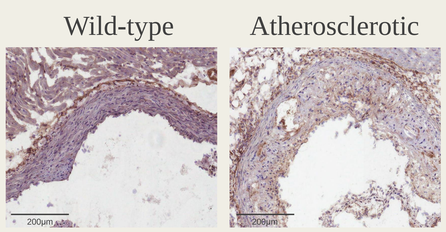
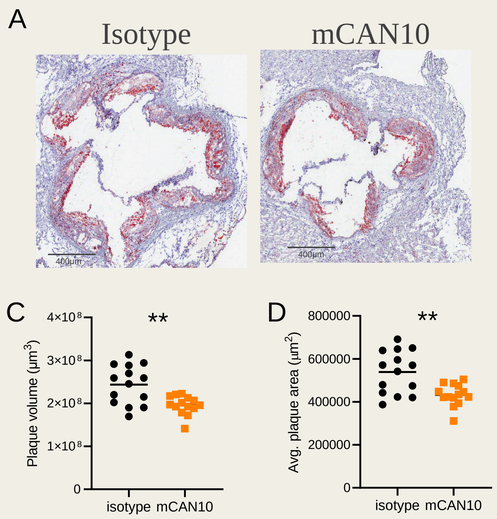


Figure 1: IL1RAP is expressed in subvalvular atherosclerotic plaques. Immunohistochemistry staining of IL1RAP in the aortic root of wild-type (C57Bl/6, no plaque) and an atherosclerotic (*Apoe*$^{-/-}$) mouse. IL1RAP staining was observed in the plaque of the *Apoe*$^{-/-}$ mouse while minimal staining was present in the media of the wild-type mouse. IL1RAP expression was also observed in the adventitia of both strains.



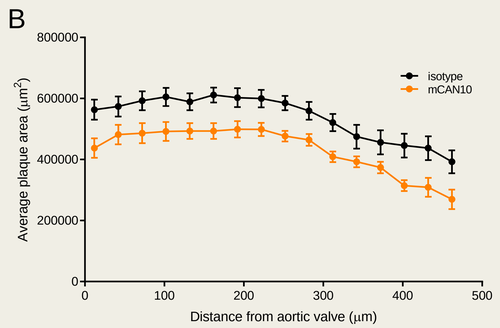
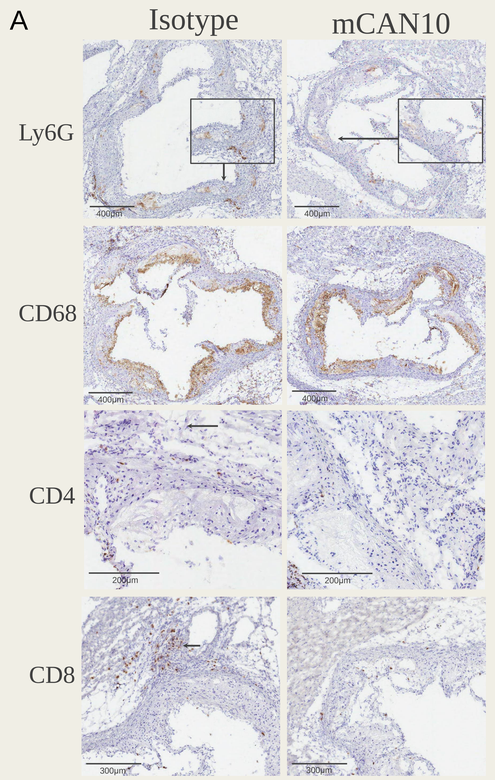


Figure 2: mCAN10 reduces plaque burden. (A) Representative lipid staining (Oil Red O) of HCD-fed *Apoe*$^{-/-}$ mice treated with mCAN10 or isotype. (B) Quantification of average subvalvular plaque area progressing through the aortic valve. Dots denote average of all mice in each treatment group at indicated distance within the aortic valve. Quantification of (C) plaque volume and (D) plaque area. mCAN10 treatment reduces plaque volume and area by an average of 20%. Bars denote mean; analysed with students unpaired *t* test. *p<0.05, **p<0.01.



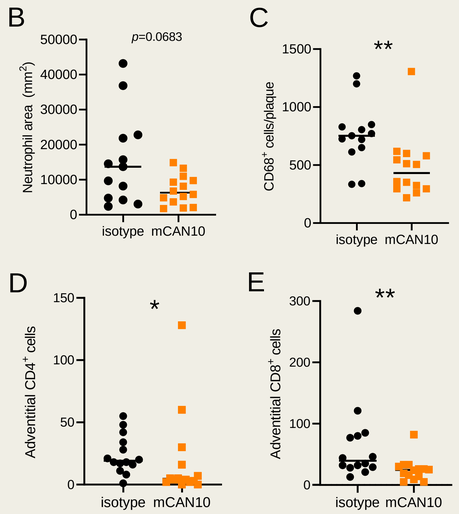


Figure 3: Treatment with mCAN10 reduces neutrophils and macrophages in atherosclerotic plaques and CD4$^+$ and CD8$^+$ T cells in adventitia. (A) Representative images of subvalvular plaque staining of HCD-fed *Apoe*$^{-/-}$ mice treated with mCAN10 or isotype. Plaques were stained for Ly6G (neutrophils), CD68 (macrophages), CD4, and CD8. Quantification of plaque-resident (B) neutrophils (Ly6G$^+$) and (C) macrophages (CD68$^+$), and (D) adventitial CD4$^+$ T cells and (E) CD8$^+$ T cells. Adventitia defined as 200 μm outwards from plaque borders. Bars denote median; analysed with Mann-Whitney *U* test. *p<0.05, **p<0.01.

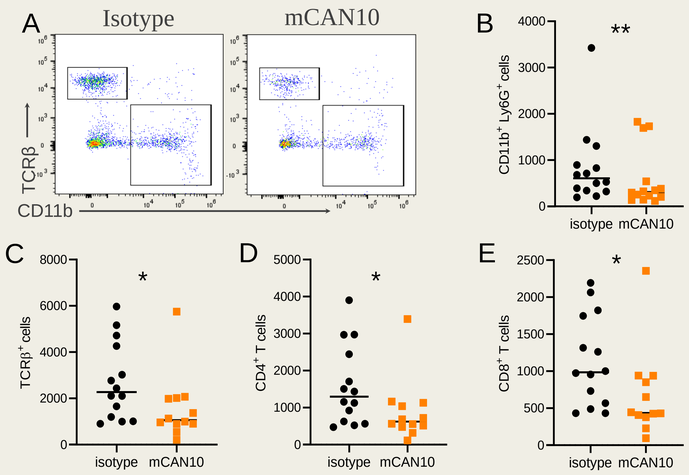


Figure 4: mCAN10 reduces numbers of leukocyte populations, including neutrophils and CD4$^+$ and CD8$^+$ T cells, in aortic digests. (A) Representative FACS plots of aortic digest of HCD-fed *Apoe*$^{-/-}$ mice treated with mCAN10 or isotype (gated on live CD45$^+$ cells). (B-E) Quantification of cell numbers in aortic digest of HCD-fed *Apoe*$^{-/-}$ mice treated with mCAN10 or isotype by flow cytometry. Bars denote median; analysed with Mann-Whitney *U* test. *p<0.05, **p<0.01.

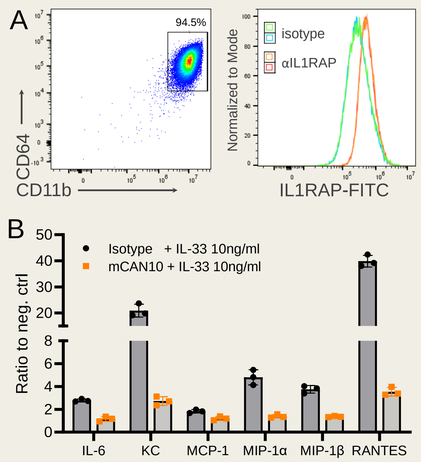


Figure 5: mCAN10 reduces production of neutrophil- and T cell-attracting chemokines from IL-33 stimulated macrophages. Bone marrow-derived macrophages (BMDM) were isolated and cultured from wild-type (C57Bl/6) mice and stimulated with IL-33 with mCAN10 or isotype control for 16hrs. (A) Flow cytometry of expression of IL1RAP on BMDMs (gated on live cells; CD11b$^+$CD64$^+$). (B) 10 ng/ml IL-33 induces production of several cytokines and chemokines by BMDMs, which can be blocked by mCAN10. Bars denote mean. Data is representative of two separate experiments.

Conclusions

Disruption of IL1RAP signalling via administration of the mCAN10 antibody reduces plaque burden and inflammation in atherosclerotic mice. Our findings support IL1RAP as a therapeutic target for limiting plaque inflammation in patients with cardiovascular disease.