Stimulation of the PD-1 pathway decreases atherosclerotic lesion development in LDLr$^{-/-}$ mice

H.W. Grievink[1,2], D. Smeets[1], N. Benne[1], R. Verwilligen[1], M. Moerland[2], J.Kuiper[1], I. Bot[1], A.C. Foks[1]

1. Division of BioTherapeutics, LACDR, Leiden University, Leiden, the Netherlands
2. Centre for Human Drug Research, Leiden, the Netherlands

INTRODUCTION

Cardiovascular disease is the number one cause of death worldwide. The major underlying cause is atherosclerosis, which is characterized by the accumulation of lipids and immune cells in the arterial wall. The local ongoing pro-inflammatory immune response leads to atherosclerotic plaque progression and ultimately destabilization, potentially resulting in acute cardiovascular syndromes such as a myocardial infarction or stroke. Immune checkpoint inhibitors, such as programmed cell death protein (PD)-1, play an important role in regulating the immune response. The ligands of PD-1, PD-L1 and PD-L2, are expressed on several immune cells, such as regulatory T cells, B cells and macrophages, and dampen the immune system. In atherosclerosis, impaired PD-1 signaling leads to aggravation of the disease. We thus hypothesized that stimulation of PD-1 has an immunosuppressive effect, thereby reducing atherosclerosis.

AIM

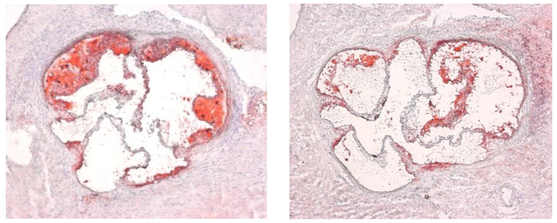
To determine whether stimulation of PD-1 results in a reduction in atherosclerotic lesion development.

EXPERIMENTAL SETUP

Western-type diet fed female LDLr$^{-/-}$ mice were treated with an agonistic PD-1 monoclonal antibody (100µg, PIM-2, twice a week) or control (PBS) for 6 weeks (n=15/group).

After 6 weeks the mice were sacrificed, leukocyte subsets (blood, spleen, HLN, PC) were assessed with flow cytometry and atherosclerosis was quantified in the three-valve area of the aortic root.

RESULTS



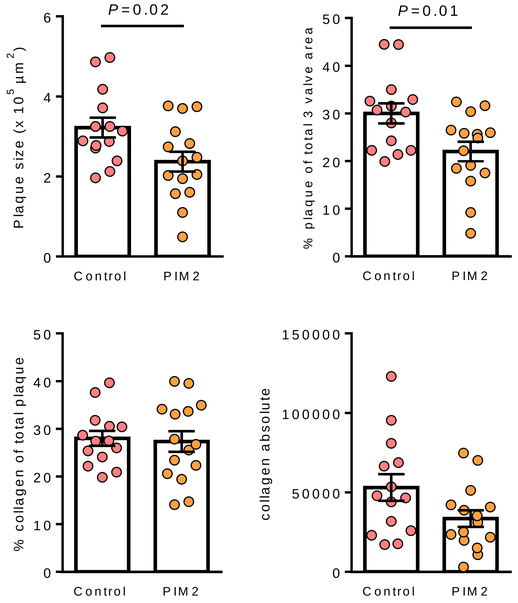


Figure 1: PD-1 stimulation (PIM2) decreases plaque size, but does not affect plaque stability. Oil Red O staining (top) of representative images of each group. Plaque size quantification (middle) based on Oil Red O staining, and collagen content (bottom) based on Trichrome staining of the three-valve area of the aortic root.

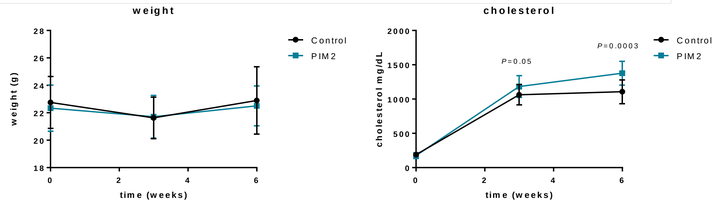


Figure 2: PD-1 stimulation did not have an effect on the weight of the mice (left), but did cause a minor but significant increase in serum cholesterol levels (right).

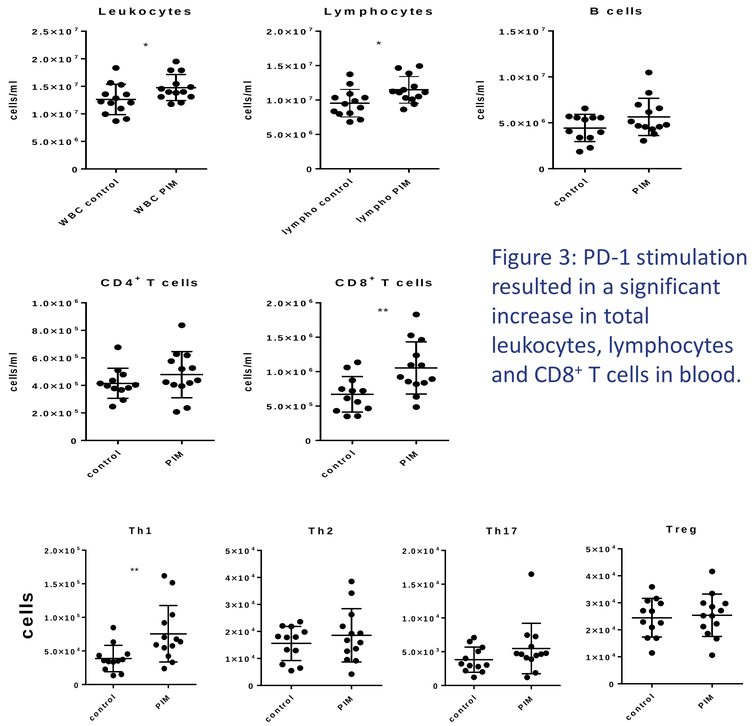


Figure 3: PD-1 stimulation resulted in a significant increase in total leukocytes, lymphocytes and CD8$^+$ T cells in blood.

Figure 4: PD-1 stimulation resulted in a significant increase CD4$^+$ T helper 1 cells in blood

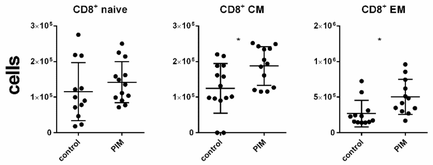


Figure 5: PD-1 stimulation resulted in an increase in CD8 effector memory cells.

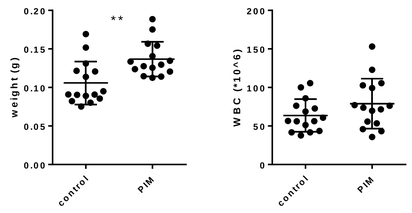


Figure 6: PD-1 stimulation resulted in an increased weight of the spleen, but not in an increased leukocyte count.

CONCLUSIONS

Stimulation of PD-1 with the agonistic antibody PIM-2 results in a decrease of atherosclerotic lesion development in LDLr$^{-/-}$ mice receiving a western type diet.

Total numbers of leukocytes, lymphocytes, Th1 cells, CD8$^+$ EM cells and spleen weight are increased after PIM-2 administration. These results do not explain the decrease in atherosclerotic lesion size, therefore further investigation is necessary.

Email: wgrievink@chdr.nl Web: www.lacdr.nl Phone: +31-071 7517160