Deficiency of the purinergic receptor P2X4 limits atherosclerosis in mice

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Introduction: Extracellular nucleotides like ATP promote inflammation as danger signals in various chronic diseases via purinergic receptors. In our previous work we identified $P2X_4$ expression in murine atherosclerotic lesions. Therefore, we hypothesized a contribution of the ATP-$P2X_4$ axis to vascular inflammation in atherosclerosis.

Methods: To investigate the functional role of $P2X_4$ in atherogenesis, wildtype LDL-receptor deficient mice (LDLR–/–) and $P2X_4$-deficient LDLR–/– mice ($P2X_4$–/–LDLR–/–) were fed a high cholesterol diet for 16 weeks. Plaque progression in aortic arches was monitored by echography at intervals of 4 weeks, and leukocyte subsets in blood samples were analysed by flow cytometry. Atherosclerotic lesions were then assessed histologically in aortic root, arch, and abdominal aorta. In order to assess leukocyte recruitment, intravital microscopy was performed after injection of ATP in $P2X_4$–/– or wildtype mice (WT). Regarding transferability to human disease, atherosclerotic plaque from carotid endarterectomy has been stained immunohistochemically for $P2X_4$-receptor expression.

Results: After 16 weeks, $P2X_4$-deficient mice showed significantly reduced atherosclerotic lesions in the aortic root (n=40, LDLR–/–: 0.47 mm^2, $P2X_4$–/–LDLR–/–: 0.39 mm^2, p=0.04). Ly6C- monocyte count in peripheral blood was higher in $P2X_4$–/–LDLR–/– (n=32, LDLR–/–: 241/μl, $P2X_4$–/–LDLR–/–: 542/μl, p=0.0088), shifting the balance to a more anti-inflammatory subset. Memory-cell generation of CD4-T-cells is significantly higher in knockout-mice, suggesting an involvement of T-helper cells (n=25, LDLR–/–: 27%, $P2X_4$–/–LDLR–/–: 46%, p=0.0003). Peritoneally injected ATP induced leukocyte rolling in WT, but not in $P2X_4$-deficient mice. In human carotid arteries, atherosclerotic plaque shows higher staining for $P2X_4$–/– receptor than not diseased areas.

Conclusion: $P2X_4$-deficiency enhances anti-inflammatory leukocytes in peripheral blood and reduces atherosclerosis. Therefore, blocking the ATP-$P2X_4$ axis may prevent leukocyte recruitment to atherosclerotic lesions and could present a potential new target for anti-atherogenic therapy.