areas of the thoracic aortas of three independent ApoE-deficient mice, and cultured in DMEM supplemented with 10% FCS. Differential gene expression of P- and NP-lines was analyzed by differential display. Functional analysis was performed by transfection of differentially expressed genes into VSMC. **Results:** Several genes that are up- or down-regulated in P-lines compared with NP-lines were identified by differential display. Sequence analysis revealed that among these genes were two novel genes with unknown function. Application of 5' rapid amplification of cDNA ends revealed that one of these genes is coding a novel peptide. Transient expression of this gene induced apoptosis in cultured VSMC, which showed condensed and fragmented nucleus by staining with Haechst. **Conclusion:** The expression of the novel gene cloned in this study may contribute to the apoptosis of VSMC in ahterosclerosis.

OJ-189

Transplantation of Matrix Metalloproteinase-2-deficient Bone Marrow Inhibits Atherosclerotic Lesion Formation in Low Density Lipoprotein Receptor-deficient Mice

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Background: Because atherogenesis is initiated by invasion of monocytes and lymphocytes into the vascular wall, matrix metalloproteinase-2 (MMP2) is thought to play a crucial role in atherogenesis. Thus, we examined the role of MMP2 in atherogenesis using bone marrow transplantation (BMT) technique. **Methods**: Low density lipoprotein receptor-deficient ($LDLR^{-/-}$) mice were lethally irradiated and MMP2-deficient bone marrow cells were transplanted ($MMP2^{-/-}$ BMT mice). $LDLR^{-/-}$ mice reconstituted with the bone marrow from $MMP2^{+/+}$ wild-type mice were used as control ($MMP2^{+/+}$ BMT mice). These mice were fed a high fat diet (HFD) for 6 weeks. **Results**: Neither survival rate nor body weight differed between $MMP2^{+/+}$ BMT and $MMP2^{-/-}$ BMT mice. Plasma total cholesterol levels were significantly higher in $MMP2^{+/+}$ BMT mice than $MMP2^{-/-}$ BMT mice. Plasma lipoprotein profile analyses revealed that LDL fraction was significantly higher in $MMP2^{+/+}$ BMT mice. The atherosclerotic lesions of $MMP2^{-/-}$ BMT mice were smaller than those of $MMP2^{+/+}$ BMT mice at the aortic sinus, aortic arch and abdominal aorta. MOMA2 (a marker for macrophages) and Oil red-O staining showed that the accumulation of lipid-laden macrophages was significantly decreased in $MMP2^{-/-}$ BMT mice. **Conclusions**: Transplantation of MMP2-deficient bone marrow cells into $LDLR^{-/-}$ mice resulted in suppression of atherosclerotic lesions. However, the mechanism by which plasma cholesterol was lowered in $MMP2^{-/-}$ BMT mice is still unclear.

OJ-190

Macrophage Specific Overexpression of Allograft Inflammatory Factor-1 in Transgenic Mice Promotes Phagocytotic Activity Following Thioglycollate Stimulation and Regulate Atherosclerotic Progression

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Macrophage Specific Overexpression of Allograft Inflammatory Factor-1 in Transgenic Mice Promotes Phagocytotic Activity Following Thioglycollate Stimulation and Regulate Atherosclerotic ProgressionTetsuya Mishima, Kazuya Iwabuchi, Satoshi Fujii, Keiko Watano, Yukihito Nakai, Akira Kitabatake, Kazunori OnoeHokkaido UniversityAllograft inflammatory factor-1 (AIF-1), originally identified in cardiac allografts with chronic rejection, is also expressed in infiltrating macrophages of the grafts. We have previously shown that a mouse macrophage cell line (RAW264.7) transfected with AIF-1 produced more atheroprotective cytokine (IL-10) than vector controls upon stimulation with lipopolysaccharide. Moreover, the transfectants exhibited enhanced phagocytotic activity. To elucidate the role of AIF-1 in vivo, we established AIF-1 transgenic (Tg) mice that express AIF-1 in $CD11b^{+}$ macrophages. The phagocytotic activities of resident peritoneal exudate cells (PEC) obtained either from Tg or non-Tg mice were examined using FITC-conjugated latex beads by flowcytometry analysis. Under baseline condition PEC from Tg-mice showed non-significant phagocytotic activity. However, markedly increased phagocytotic activity was observed in Tg-mice as compared with non-Tg upon stimulation of macrophages with peritoneal administration of thioglycollate broth ($33.2 \pm 2.3\%$:Tg vs $24.3 \pm 2.9\%$:non-Tg, n=5 , $p<0.05$). Phagocytotic abilities of activated macrophages from Tg-mice were closely correlated with the degree of AIF-1 expression. Upon immunohistochemistry macrophages positive for AIF-1 were found in atherosclerotic lesions. Thus, AIF-1 may induce atheroprotective cytokine production and phagocytotic activity in activated macrophages, hence retard atherosclerotic progression.

OJ-191

GTPCH I Overexpression Decreases Atherosclerotic Lesion Formation in Apolipoprotein E-Deficient/eNOS Transgenic Mice

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Background: We reported that eNOS overexpression accelerates atherosclerotic lesion formation by crossing apoE-deficient mice (apo E-KO) with eNOS transgenic mice (eNOS-Tg). As the mechanisms, we showed that there are insufficient levels of vascular tetrahydrobiopterin (BH4) in these mice, which result in eNOS dysfunction. Dysfunctional eNOS produces superoxide rather than NO and accelerates atherogenesis. In the synthesis BH4, GTP-cyclohydrolase I (GTPCH) plays a pivotal role as the rate-limiting enzyme for its *de novo* biosynthesis. In this study, we examined whether GTPCH overexpression in the endothelium inhibits the development of atherosclerosis in eNOS-overexpressing apoE-KO mice (apo E-KO/eNOS-Tg). **Methods and Results:** We crossed apoE-KO/eNOS-Tg with apoE-KO/GTPCH-overexpressing mice (GTPCH-Tg) and fed a normal chow for 16 weeks. Body weight, lipid contents and blood pressure were not significantly different among ApoE-KO, ApoE-KO/eNOS-Tg, and ApoE-KO/eNOS-Tg/GTPCH-Tg. The atherosclerotic lesion areas in the aortic root were increased apoE-KO/eNOS-Tg compared with apoE-KO as we reported previously. Overexpression of GTPCH reduced the lesion size in apoE-KO/eNOS-Tg to the levels comparable to apoE-KO. This reduced lesion size was associated with decreased superoxide production from vessels. **Conclusion:** Overexpression of GTPCH reduced the atherosclerotic lesion formation of apoE-KO/eNOS-Tg mice. This study shows the importance of endogenous BH4 in atherogenesis, and may imply the potential therapeutic strategy for treatment of atherosclerosis.

OJ-192

Angiotenisin II type 1a receptor-deficiency reduced the expression of matrix metalloproteinase in atherosclerosis of apoE-deficient mice

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Background We reported that angiotensin II type 1 receptor (AT1)-deficiency reduced atherosclerosis and extracellular matrix (ECM) production in apolipoproteinE-defecient mice (apoE-/-). There is a possibility that decreased ECM production causes plaque instability of atherosclerosis. Therefore, we investigated whether AT1-deficiency affected plaque instability of atherosclerotic lesion using AT1a-deficient mice (AT1a-/-) and apoE-/-. **Methods** AT1a-/- crossbred with apoE-/-, and homozygous knockout mice for AT1a (AT1a-/-/apoE-/-) and wildtype mice at AT1a locus (AT1a+/+/apoE-/-) were established. Male mice were fed a chew diet and analyzed at 60 weeks of age. Azan staining for collagen and Victoria blue staining for elastin were performed to assess the matrix production. Immunohistochemistry for matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) which related to plaque stability, and C-reactive protein (CRP) which related to inflammation, was performed. **Results** The atherosclerotic lesions of AT1a-/-/apoE-/- mice were significantly smaller than that of AT1a+/+/apoE-/-. The amount of collagen and elastin of AT1a-/-/apoE-/- was also less than that of AT1a+/+/apoE-/-. The immunoreactivity of MMP-2, MMP-9, and CRP in AT1a-/-/apoE-/- was less than that in AT1a+/+/apoE-/-. These results suggested that the inhibition of MMP activity and CRP production by AT1a-deficiency facilitated plaque stability in atherosclerotic lesion. **Conclusion** Although AT1a-deficiency reduced ECM production, it also inhibited MMP activity and inflammation. These results support the effect of AT1 blockade on plaque stability of atherosclerotic lesion in clinical use.