Methods: VSMC grown in serum free media for 48 hours were co-cultured with mono-
cytes (M) for 12 hours, in the presence of M-CSF or IL-1β. The apoptotic index was mea-
sured using an established apoptosis assay. M-CSF and IL-1β were used at con-
centrations of 100ng/ml and 100ng/ml to 1ng/ml, respectively. Appropriate controls 
were set up for all experiments.
Results: L.1β at physiological concentrations (100ng/ml) did not increase VSMC apopto-
sis. However, L.1β + M-CSF induced significantly increased apoptosis (60.6±3.0, ps = 0.008).
Supraphysiological doses of L.1β (500ng and 100ng/ml) did cause an increase in mono-
cyte-induced killing of VSM (56.5±3.5 and 59.0±3.6 respectively). However, such effect 
was shown to be mediated by endogenous production of M-CSF upon IL-1β stimu-
lization. Thus, VSMC co-cultured with M and L.1β (500ng and 1ng/ml) were pretested 
with an anti-M-CSF neutralizing antibody (IgG1). The VSMC apoptosis was nearly abro-
gaged in the presence of the anti-M-CSF Fab (17.0±2.1% and 13.6±1.3%, p<0.007 and 
p<0.006 respectively), when compared to VSMC co-cultured with M-CSF activated M 
alone. A nonspecific isotype control (IgG3a) was used and did not block the effect 
of L.1β on induced VSMC apoptosis (56.0±2.0%).
Conclusions: M-CSF could function as an important rate limiting cytokine in the process of activated monocyte induced killing of VSMC, suggesting a final common pathway for atheroma plaque 
destabilization.


853-5 Arterial Injury in ApoE –/– and C57/B16 Wild Type Mice: Evidence for Macrophage Apoptosis as a Mechanism Regulating Tissue-Factor Expression and Plaque Growth

Randolph Hutter, Juan J. Badimon, Bernhard V. Sauter, John T. Fallon, Valentin Fuster, Cardiovascular Institute, Mount Sinai Medical Center, New York, New York.

Background: Macrophages undergoing apoptosis are considered contributing to pro-
gression of atherosclerotic lesions. Tissue factor is a key cell-mediated activator of extrin-
scoagulation cascade and can induce thrombus formation.

Methods: In the present study, we examined the role of macrophage apoptosis on 
plaque growth by comparing neointima formation in ApoE –/– (n=10) and C57/B16 wild 
type mice (n=23) in a model of femoral arterial denudation injury

Results: Arterial injury resulted in significantly increased neointima formation in ApoE –/– 
mice (6.5±1.5 mm2 x 106 vs. 0.73±0.03 mm2 x 106; P<0.01). Apoptotic macrophages and foam cells as detected by caspase-3 expression, characteristic morphology and positive staining for MOMA-2 were found only in lesions of ApoE –/– mice and accounted for up to 33 ± 6 % of intimal cells at 4 weeks after 
arterial injury compared to less than 0.5 % apoptotic macrophages in neointima of wild type 
mice (P<0.01). Importantly, neointima size significantly correlated with the content of apo-
ptotic macrophages in neointima (r=0.64, P<0.01). In addition, apoptotic macrophages 
were directly associated with increased cellular tissue factor expression (r=0.97, P<0.01).

Conclusions: Apoptosis of macrophages and enhanced expression of cellular tissue factor 
in neointima correlated with increased plaque growth and change of neointima to an unstable plaque-like phenotype. These findings point to an important role of pro-
grammed cell death occurring in macrophages in modulating arterial lesion biology and 
controlling thrombogenic properties of intimal lesions following arterial injury.

11:45 a.m.

853-6 Asymmetric Dimethylarginine Stimulates the Expression of Matrix Metalloproteinase-9 in Human Monocytes via Tumor Necrosis Factorα Factor-a

Eun-Myoung Jeong, Sangyoui Hong, Jeong-Euy Park, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea, Center for Clinical Research, Samsung Biomedical Research Institute, South Korea.

Background: Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibi-
tor of nitric oxide synthase, has recently been reported as an atherogenic molecule. ADMA induces the endothelial dysfunction and influences the monocyte adhesion to the 
endothelial cells. We tried to investigate whether ADMA induces the release of proinflem-
atory cytokines and the expression of matrix metalloproteinase-9 (MMP-9), chemotatic factors such as IL-8 and monocyte chemoattractant protein (MCP)-1. No

Results: ADMA induces the endothelial dysfunction and influences the monocyte adhesion to the 
endothelial cells. We tried to investigate whether ADMA induces the release of proinflem-
atory cytokines and the expression of matrix metalloproteinase-9 (MMP-9), chemotatic factors such as IL-8 and monocyte chemoattractant protein (MCP)-1. No

Conclusions: This study suggests that ADMA induces the release of pro-inflammatory cytokines, TNFα, IL-8 and MCP-1 in monocytes. The up-regulation of MMP-9 is mediated by TNFα in ADMA treated mono-
cytes. These findings provide the evidences that ADMA may be involved in the plaque formation and destabilization in atherosclerosis.

Poster Session

1176 Nitric Oxide, Smoking, and Vascular Function

Tuesday, March 19, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: Noon-1:00 p.m.

1176-69 Effects of Low Dose Hormone Replacement Therapy on Nitric Oxide Bioactivity and Markers of Inflammation Compared With Conventional Dose in Postmenopausal Women

Kwang K. Koh, Mi-Seung Shin, Gachon Medical School, Incheon, South Korea.

Background: We have previously shown that conventional dose hormone replacement 
therapy (C-HRT) improved nitric oxide (NO) bioactivity and reduced markers of inflam-
ination in postmenopausal women (PMW). The effects of low-dose hormone replace-
ment therapy (L-HRT) has not yet observed.

Methods: We administered micronized progesterone (MP) 100 mg with conjugated 
equine estrogen (CEE) 0.625 (C-HRT) or 0.3 (L-HRT) mg daily for 2 months to 15 PMW 
with a washout period of 2 months In a randomized, double-blind, crossover study. Data=
mean±SD. *p<0.05. **p<0.01 vs. Baseline.

Results: L-HRT and C-HRT significantly changed lipoprotein levels. L-HRT improved 
flow-mediated dilatation (FMD) and tended to reduce plasma E-selectin, but not VCAM-1, 
CRP and fibrinogen levels compared with respective baseline levels. However, there 
were no significant differences between C-HRT and L-HRT regarding these effects. There 
were no significant correlations between absolute levels or percent changes of 
CRP levels and absolute levels or percent changes of FMD or E-selectin (all r<0.15).

Conclusion: L-HRT has comparable effects to C-HRT in PMW regarding lipoproteins, 
FMD, and markers of inflammation.

1176-71 Cyclosporine-induced Hypertension and Vascular Superoxide Production

Joseph N. Graziano, Carolyn L. Buier, John R. Charpie, University of Michigan, Ann Arbor, Michigan.

Background: Cyclosporina (A) -induced endothelial dysfunction and systemic hypertension contribute to significant morbidity and mortality following orthopeic heart 
transplantation. Several experimental models of hypertension have recently been linked 
with endothelial dysfunction and increased vascular oxidant stress. We hypothesize that 
Cyclosporin increases vascular superoxide (O2-) production leading to a decrease in bioavail-
able nitric oxide, an endogenous vasodilator.

Methods: Adult male C57BL/6J mice (age 10-12 weeks) were treated for 12 days with 
either CEA (25 mg/kg in olive oil iv intraperitoneal injection, n=12), vehicle (n=5), 1 mm 
Temol (a superoxide dismutase mimetic administered in the drinking water; n=5) or CSA + 
Temol (n=5). Systolic blood pressure (SBP) was measured daily by tail cuff method.

Additionally, cultured human umbilical vein endothelial cells (HUVEC) were grown to 
fusion and incubated in phosphate buffered saline with 0.1 μM CSA (or ethanol vehicle) 
for ten minutes. Superoxide production was measured (μM) by lucigenin-dedaved chemi-
luminescence (5 μM).

Results: CSA administration resulted in a significant increase in SBP from 113±5 mmHg 
(mean ± SEM) treatment to 184±2 mmHg on day 12 (p<0.001). In contrast, co-
administration of Temol with CSA completely inhibited the CSA-induced increase in 
SBP (111±2 mmHg on day 12). Neither vehicle nor Temol alone had any effect on SBP. 
Incubation of HUVEC with CSA resulted in a 135±15% increase in O2- production com-
pared to HUVEC alone (p<0.02). Incubation of HUVEC with the endothelial nitric oxides 
synthase inhibitor N°-monomethyl L-arginine (L-NMMA, 1raM) resulted in an 85±10% 
increase in O2- production (p=0.04). Furthermore, there was a synergistic response to L-
NMMA and CSA with an 85±10% increase in O2- production compared to HUVEC alone 
(p<0.001). Tempol (1 mm) completely inhibited the increase in O2- in both CSA and 
CSA + L-NMMA treated cells.

Conclusions: CSA treatment increases blood pressure and endothelial cell O2- produc-
tion. Inhibition of nitric oxide production augments the increase in CSA-induced O2-
production, suggesting that excess O2- may scavenge nitric oxide and decrease its 
bioavailability.