VIII

Conclusion
**VIII. Conclusions**

In the present study, expression and localization of the transcription factors: IRF-1 and IRF-2 and expression of their target genes were investigated during lipopolysaccharide (LPS)-induced inflammation in the mouse liver, which showed that IRF-1 and IRF-2 genes were sensitive and responsive to the inflammatory agent *in vivo*. An experimental mouse model for the high fat diet (HFD)-induced atherosclerotic lesions in the aorta was established to study possible involvement of IRF-1 and IRF-2 in atherosclerosis. Effect(s) of two possible therapeutic agents, i.e., Curcumin and all trans-Retinoic Acid (atRA), on the development of aortic atherosclerotic lesions, expression of IRF-1, IRF-2 and their target genes was examined. Finally, effect of Curcumin on *in vitro* DNA-binding activity of recombinant GST-IRF-1 fusion protein was examined.

The following are the conclusions from the data presented in this study:

1. Expression of IRF-1, IRF-2 mRNAs was induced in response to systemic administration of low dose of endotoxin, LPS (2.5 to 20 μg per 25 g body weight) within 2 hours in the mouse liver. This may be correlated to the early phase of bacterial infection when the pathogenic molecule in circulation is low. IRF-2 mRNA expression was induced possibly by pre-existing and activated IRF-1 protein at an earlier time point than the induced IRF-1 mRNA expression. Both IRF-1 and IRF-2 proteins were localized to the nucleus in the LPS-treated liver cells. Further, a marked increase in the expression of the IRF-1 and IRF-2 target genes: cyclooxygenase-2 (COX-2), monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase-9 (MMP-9) and the cyclin-dependent protein Kinase Inhibitor, p21 was observed, indicating inflammation, immune cell infiltration, tissue remodelling and cell growth arrest in the liver mediated through IRF-1 and IRF-2. Thus, IRF-1 and IRF-2 are induced within two hours in response to the inflammatory stimuli and regulate expression of the downstream genes.

2. A high fat diet (HFD)-induced experimental atherosclerosis model was established in the female C57BL/6J mice. The composition of HFD, used in this study, was based on a published atherogenic diet (Paigen B et al. 1987) and the mice fed with this diet developed reproducible fatty lesions at the aortic root.
Expression of IRF-1, IRF-2 mRNAs was induced in the aorta under atherogenic conditions. A significant increase in the mRNA expressions was observed for IFN-β, MCP-1, VCAM-1 (vascular cell adhesion molecule-1) and MMP-9 genes in the aorta of HFD-fed mice. These IRF-1 target genes induce inflammatory progression of aortic lesions indicating a possible role for IRF-1 in mediating the lesion formation. IRF-2 may also participate in the expression of these genes and development of the lesion.

3. Curcumin administered along with HFD at low and high dose (1.2 and 2.5 mg/mouse/day) lowered the area of aortic lesions by ~46% and 29%, respectively. Curcumin showed varied effects on expression of IRF-1 and IRF-2 with respect to the dose and the diet. Low dose Curcumin induced IRF-1 mRNA and protein, while high dose Curcumin induced IRF-1 protein but not IRF-1 mRNA in both control and high fat diets. Expression of IRF-2 mRNA and protein was induced by low dose Curcumin in control diet but remained unaltered in HFD. High dose Curcumin induced expression of IRF-2 protein but not mRNA in control diet, however, it decreased expression of IRF-2 mRNA but not IRF-2 protein in high fat diet. Increase in IRF-2 protein in the absence of mRNA induction may be due to increased protein stability. Protein localization by immunohistochemistry showed that IRF-1 and IRF-2 proteins were concentrated at the luminal surfaces of the aortic lesions. Low dose Curcumin treatment with HFD induced expression of HO-1 (Heme Oxygenase-1), IDO (Indoleamine 2,3-dioxygenase), COX-2, MMP-9 mRNAs. High dose Curcumin treatment with HFD induced expression of MCP-1 and decreased MMP-9 and LOx (Lysyl oxidase) mRNAs.

4. atRA with HFD also reduced aortic lesion area by 23% at low dose (1.4 μg/mouse/day) and 14% at high dose (2.5 μg/mouse/day), respectively. However, it was not as effective as Curcumin. atRA also induced small accumulations of foam cells in the aortic wall of mice fed with control diet. atRA at both doses significantly induced expression of IRF-1 mRNA and protein in both control and HFD. Expression of IRF-2 mRNA was induced by low and high dose atRA in control diet but remained unaltered in HFD. Expression of IRF-2 protein was induced by low and high dose atRA in control diet but decreased by high dose.
Conclusions

atRA in HFD. High dose atRA treatment with control diet significantly induced expression of IFN-β, MCP-1, VCAM-1, MMP-9, HO-1, IDO and p21 mRNAs; the same treatment with HFD only induced expression of LOx, HO-1 and IDO.

5. Treatment of a combination of low dose Curcumin and low dose atRA induced IRF-1 mRNA and protein expression in both control and HFD. The combination treatment in HFD decreased expression of IRF-2 protein but the mRNA remained unaltered. Low dose Curcumin in combination with low dose atRA in control diet induced IFN-β, VCAM-1 and p21 mRNAs but with HFD induced COX-2, VCAM-1, MMP-9, HO-1 and IDO mRNA expression. However, this treatment did not reduce aortic lesion area.

6. Analysis of the in vitro DNA-binding showed that 10 μM Curcumin inhibited DNA-binding activity of 50 nM GST-IRF-1 up to 30%. Direct inhibition of the DNA-binding activity of IRF-1 by Curcumin and the mechanism of interaction between Curcumin and GST-IRF-1 needs further investigation.