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Statement of the Problem
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Interferon Regulatory Factors, IRF-1 and IRF-2 are members of a family of transcription factors with pleiotropic roles in mammalian cells. These factors regulate Interferon (IFN) and IFN-responsive genes during innate immune response as well as have much broader physiological role, including regulation of cellular immune response against pathogens, differentiation of cells in the immune system, differentiation of cells during embryonic development, maturation of immune cells, regulation of cell growth, apoptosis and tumorigenesis. IRF-1 and IRF-2 have been shown to have antagonizing roles in regulating certain genes involved in inflammation (e.g., IFN-β, COX-2, iNOS, MCP-1, VCAM-1, Caspase-1, etc) and they also independently regulate certain other genes associated with cell growth regulation/arrest (e.g., IRF-1: p21WAF1/CIP1, IRF-2: histone H4), apoptosis (e.g., IRF-1: caspase-1, TRAIL; IRF-2: FasL), cell adhesion, migration (e.g., IRF-1: MMP-9, LOx, VCAM-1) and others. IRF-1, IRF-2 regulated genes mediating inflammatory processes are also triggered by pathogenic stimuli (e.g., LPS and dsRNA). It is now widely accepted that the persistence of inflammation and immune response may lead to several chronic diseases including atherosclerosis, arthritis, liver cirrhosis, hepatic steatosis, diabetes and certain types of cancers. Thus, a key role for IRFs, particularly IRF-1 and IRF-2, is expected in the pathogenesis of such diseases and the same is reported in case of cervical and breast cancers and diabetes. However, their role in atherosclerosis has not been studied, except for a report published by Wessely R et al. (2003), which demonstrated that adenoviral delivery of IRF-1 inhibited neointimal growth in mouse in vivo. Since IRF-1 and IRF-2 are key regulators of inflammation, cell growth, apoptosis and cell migration, they may help understanding the molecular pathogenesis of atherosclerotic lesions and may define candidate therapeutic targets for the disease. Effect of certain known/possible therapeutic agents such as Curcumin and all trans-Retinoic Acid (atRA) may also be studied in this context.
With this background following hypothesis was proposed:

- The expression and/or activity of IRFs (e.g., IRF-1 and IRF-2) may be altered in response to systemic administration of inflammatory stimuli and these factors may then regulate the expression of genes involved in the cellular inflammatory processes in mouse tissues.

- Since atherosclerosis is recognized as a chronic inflammatory disease to begin with, expression of IRF-1 and IRF-2 may be induced or activated in the aorta with atherosclerotic lesions, where they may mediate the inflammatory processes by regulating their downstream target genes. In such case, IRF-1 and IRF-2 may prove to be important either as cellular markers or as therapeutic targets in aortic atherosclerosis.

To address the above questions/hypothesis, following objectives were designed:

- First, expression and trans-activation function of IRF-1 and IRF-2 in mouse liver after systemic treatment with low dose(s) of an inflammatory agent, the bacterial endotoxin: lipopolysaccharide (LPS) was checked. LPS is an example of pathogen associated molecular pattern (PAMP) derived from the cell wall of gram negative bacteria and is recognized by the pattern recognition receptors, Toll-like Receptors (TLRs) present on immune cells. Low doses (1 to 20 μg) of systemic LPS-administration and relatively short treatment time-periods (0.5 to 6 h) were selected to mimic the early phase of cellular response to the infection. Since liver is a prime site for metabolism of toxic agents, mixing of the blood and clearance of pathogens, hepatic IRF-1 and IRF-2 may respond to such agents, if they are involved in the inflammatory process.

- In order to study the role of IRF-1 and IRF-2 in aortic atherosclerosis, we sought to establish a high fat diet (HFD)-induced experimental atherosclerosis model in the mouse. The C57BL/6J mouse strain was fed with the atherogenic diet (also
known as the “Paigen diet”) to develop fatty streak lesions at the aortic root. This should represent an inflammatory vascular pathogenesis.

- Effect of two possible therapeutic agents on the progression of atherosclerosis and expression of IRF-1, IRF-2 and their responsive genes in atherosclerotic lesions were studied. They are: (a) Curcumin, a natural polyphenolic, anti-inflammatory, antioxidant molecule with chemopreventive property, which has been tested in clinical trials for curing certain types of cancers, due to its non-toxicity even at higher doses, Curcumin appears to be an attractive therapeutic agent, the anti-inflammatory and atheroprotective activities of Curcumin may be partly mediated by IRF-1 and IRF-2; (b) all trans-Retinoic Acid (atRA), a physiological factor for immunity, cell differentiation, apoptosis and embryonic development, which also induces IRF-1 gene expression. Also, to check if the expression of IRF-1, IRF-2 and selected IRF-regulated genes, that mediate inflammation (e.g., IFN-β, MCP-1, VCAM-1, IDO), cell growth regulation/apoptosis (e.g., p21, Caspase-1, TRAIL) and matrix modification (e.g., MMP-9, LOx) are responsive to HFD-feeding as well as Curcumin and atRA supplementation in the diet.

- Small molecule regulators of key enzymes and proteins are attractive for present day drug development. Among these, small molecule regulators of transcription factors [e.g., pyrrole-imidazole polyamides for Nuclear Factor kappaB (NF-kB), phenol-furan-benzimidazole for Pit-1 and Brn-3 (POU domain interacting proteins) and Curcumin for Egr-1, Jun-Fos (AP-1)] are gaining importance. Such molecules may directly inhibit DNA-binding activity of specific transcription factors. It was proposed to check if Curcumin may influence the DNA-binding activity of IRF-1, thereby regulating the downstream processes. This was addressed by analyzing the effect(s) of Curcumin on the in vitro DNA-binding activity of recombinant GST-IRF-1 fusion protein with a synthetic promoter/enhancer sequence, (GAAAGT)$_4$. 

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