Chapter 11
Summary and Conclusion
11. **Summary and Conclusion**

- The level of inflammatory cells and inflammatory score was high in patients with low level of regulatory cells compared to control. An increase in inflammation and deficient immune regulation could be observed with disease severity.

- Autoimmune response to HSP 60 and APOB 100 was found to increase the expansion of inflammatory cells. The regulatory cells from patients were found to be functionally defective as they were unable to control the inflammatory cell expansion and foam cell formation in comparison to those from healthy controls.

- Hyperglycemia induced an ER stress in cells and expanded the inflammatory cell expansion and apoptosis in regulatory cells, suggesting a link between stress and loss of function of regulatory cells.

- The level of subclinical infection was not differentiating patient and control population but CMV and CPN infection level shows correlation to inflammatory cells.

**Overall conclusion**

In conclusion, our results suggest an important role for Th17 and Tc17 cells in acute coronary disease in the Indian population. HSP60 and Ox-LDL may contribute to this response and pathogenesis of AMI in the Indian population. Young MI patients with no apparent risk factors could be distinguished by the increase in Th17 cells and Th17/Treg ratio in peripheral blood. The ratio of Th17 to Treg cells in the peripheral blood emerged as an independent marker showing significant association with acute myocardial infarction and could be developed as a potential new marker to differentiate individuals at a higher risk for developing an acute clinical event.
Our results also suggest that hyperglycemia and smoking are major factors which increase the subpopulation of monocytes and T cells in circulation. These cells also showed a positive association with diabetes and coronary artery disease, suggesting they can be considered as markers of systemic inflammation. The other important conclusion from this result is that elevated glucose levels can induce a state of systemic inflammation which can have major pathogenic consequences as reported by a recent report in Nature communications suggesting that enhanced fermentation of glucose stimulates oncogenic potential in cells [234].

Based on the results, we hypothesized that HSP60 is expressed in endothelial cells in response to stress and induce an autoimmune response leading to Th17 expansion. In addition, the CCR2 positive monocytes secrete IL6 and help in IL23 induced expansion of Th17 cells. In the absence of immune regulation by Treg cells, there is continued secretion of pro-inflammatory cytokines, expanding inflammation and plaque destabilizing factors which promote plaque vulnerability and myocardial infarction. Reducing stress and restoring the immune inflammatory balance could be an important therapeutic strategy for cardiovascular patients. It is interesting to note that Foxp3+ regulatory cells significantly increased during event-free survival.

The immune system is being considered as a potential target for the prevention of cardiovascular diseases. The promising results of immune modulation in animal models of atherosclerosis have been shown by us and others. In this study, we observed a correlation between improvement in condition with regulatory immune cells. One limitation of this study is the lower number of samples in each group and also the samples were collected (although within 24 hours and before any interventional therapy)
after the occurrence of the event. A long-term prospective study may help in further strengthening the data towards formulating strategies involving the immune system for CVD therapy.

We have reported that the imbalance observed in Th17 and Treg in CAD is contributed by functional inactivity of Treg cells. To further validate these results purified Treg cells from healthy and affected individuals were cultured in different glucose containing media. Interestingly the presence of higher glucose concentration induced apoptosis of Treg cells as there was a significant increase in late apoptotic and necrotic cells compared to normal glucose media. These results suggest that hyperglycemia reduces the Treg cells thus increasing the Th17: Treg ratio leading to an inflammatory condition.

To understand the mechanism of hyperglycemia-induced differentiation we assessed the stress response in THP1 monocytes and in PBMC from patients and healthy individuals. Increase expression of ER stress markers in response to glucose in a time-dependent manner suggests that as the circulating cells are exposed to higher glucose there is a constant insult to the ER which tries to restore the homeostasis by activating the ER stress response. With a chronic exposure, the stress response turns to activate the pro-inflammatory cells which propagate the inflammation associated with CAD.

Hyperglycemia is one of the factors that induce endoplasmic stress in cells. The proteins expressed as a result of ER stress are involved in disease pathophysiology of atherosclerosis. The Smooth muscle cells which protect the stability and integrity of the plaque by collagen production undergo apoptosis by one of the proteins produced due to
the outcome of ER stress. Hyperglycemia is also known to induce inflammatory classical monocytes to produce proinflammatory IL6 cytokines. (Figure 11.1)

**Figure 11.1 ER stress**

![ER stress diagram](image)

Our study also suggests that serum antibody levels to HSP60 and ApoB 100 are independently associated with a lower risk of acute coronary syndrome, suggesting a protective function for these antibodies in cardiovascular disease. In healthy individuals, the protective antibodies may contribute to controlling inflammatory autoimmune response. Presence of hypertension, diabetes or smoking enhances inflammation and thus reduces the level of circulating protective antibodies. A negative correlation of these antibodies to Th17 cells and inflammatory cytokines suggests that adaptive immune response to autoantigens augments the inflammatory T cell response, which could further contribute to the susceptibility of individuals to acute coronary events.
In conclusion, the overall summary of the results from this study suggests that there is a progressive loss of regulatory T cells and expansion of inflammatory cells with severity of CAD. Stress induced by hyperglycemia is an important factor which can activate autoimmune response, inflammation and loss of Treg function, leading to expansion of inflammatory cells. This also results in loss of protective antibodies which can sequester the autoantigens and reduce the exacerbation of autoimmune response. Controlling stress and early detection and prevention of hyperglycemia will be beneficial in controlling the high prevalence of CAD in India.