I would like to thank the International Atherosclerosis Society for awarding me a 6 month Visiting Fellowship which enabled me to visit Prof. Andrew Lichtman’s laboratory in the Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School in Boston, USA. This fellowship has provided an excellent training milieu that will be of great help for my future career as an investigator in cardiovascular disease.

I worked on two projects at Dr. Andrew Lichtman’s lab:

1. The role of the PD-1/PD-L1 pathway in ischemia/reperfusion injury

Myocardial infarction due to sudden blockage of a coronary artery is one of the major causes of mortality worldwide. The most effective way to reduce heart injury soon after a heart attack is to restore blood flow (reperfusion) to damaged tissue by stents or coronary bypasses. Reperfusion provides oxygen, nutrients, and white blood cells needed for repair of the damaged heart tissue. However the restored blood flow can also exacerbate damage due the activity of white blood cells that are delivered into the tissue. The mechanisms underlying this thin line between repair and reperfusion injury are poorly understood and therefore a better understanding of these processes is key to the design of therapeutic strategies to limit morbidity and mortality after heart attacks.

During my 6-month visiting fellowship I investigated whether the PD-1/PD-L1 pathway, known to inhibit activation of certain white blood cells, plays a protective role in the myocardium during reperfusion after a heart attack. Whereas PD-1/PD-L1 are key regulators of atherosclerosis and myocarditis, their role in coronary heart disease is unclear. This is especially relevant now that numerous clinical trials explore the use of blocking anti-PD-1/anti-PD-L1 antibodies as treatment of cancer. However, in contrast to checkpoint blockade in cancer where heightened immune responses are desired, the unwanted activation of the immune system needs to be suppressed in inflammatory diseases.

Previously, research by Dr. Andrew Lichtman’s laboratory has shown that myocardium expresses significantly more PD-L1 encoding mRNA than most other tissues, and that cardiac endothelium expresses PD-L1. In addition, humans with a SNP in the PDCD1 gene exhibit a decreased risk for nonfatal myocardial infarction. Taken together, these findings lead us to hypothesize that the PD-1/PD-L1 pathway is directly involved in regulating inflammatory responses following ischemic reperfusion injury. I therefore performed LAD-ligation ischemia/reperfusion studies in genetically deficient PD-1 or PD-L1
mice in collaboration with Ronglih Liao’s laboratory at the Brigham and Women’s Hospital. Cardiac function was measured using echocardiography and inflammatory infiltrates of the hearts were investigated by immunohistochemical stainings. Preliminary studies were performed to determine the optimal time to assess inflammatory infiltrates after infarction, and the optimal position of LAD ligation to yield the least variable infarction size. Initial experiments to compare these parameters between PD-1 and PD-L1 KO vs control mice have been performed and are being analyzed. Additional experiments are scheduled.

2. **The role of Tim-1 and Tim-4 in atherosclerosis.**

I also worked on another project during my visit at Dr. Andrew Lichtman’s lab which focused on the role of Tim-1 and Tim-4 in atherosclerosis. Proteins of the transmembrane T cell immunoglobulin and mucin domain (Tim) family are expressed by numerous types of immune cells, recognize phosphatidylserine (PS) on plasma membranes and exert either a costimulatory or coinhibitory role. Tim-1 is mainly expressed by Th2 cells but can also be found on dendritic cells and some B cells. Tim-4, present on macrophages and antigen-presenting cells, has been shown to play a critical role in the clearance of apoptotic cells, regulates the number of PS-expressing activated T cells, and is genetically associated with low LDL and triglyceride levels.

Since the functions of both Tim-1 and Tim-4 could affect processes of atherosclerosis, I investigated whether modulation of Tim-1 and Tim-4 may represent a novel therapeutic approach to treat cardiovascular disease. This study showed that treatment with anti-Tim-1 or anti-Tim-4 enhanced atherosclerosis compared with control mice by affecting apoptosis and CD4⁺ T cells. Whereas anti-Tim-4 treatment increased Th1 and Th2 responses, anti-Tim-1 treatment induced Th2 responses and strongly reduced the percentage of regulatory T cells (Tregs). Furthermore, anti-Tim-4-treated mice showed increased percentages of activated T cell and 'late' apoptotic cells in the circulation. In conclusion, blockade of Tim-4 aggravates atherosclerosis likely by prevention of phagocytosis of PS-expressing apoptotic cells and activated T cells by Tim-4-expressing cells, whereas Tim-1-associated effects on atherosclerosis are related to changes in Th1/Th2 balance and reduced circulating Tregs.

**Conclusion**

In summary, this fellowship gave me an excellent opportunity to broaden my scientific expertise by learning new techniques such as echocardiography to measure cardiac function and digestion of the heart and descending aorta to analyze cell subsets with FACS. I have collaborated with several experts in the
field of immunology and cardiovascular disease, which greatly expanded my scientific network. In addition to my laboratory training, this fellowship gave me an opportunity to participate in seminars and conferences emphasizing issues pertinent to immunology and vascular biology. Most importantly, this fellowship enabled me to establish a future collaboration between my home laboratory and Dr. Andrew Lichtman’s lab.

Thank you again for funding this great opportunity to work abroad, learn new techniques and exchange valuable information between my home and host institute.

Sincerely,

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