ImmunoTools IT-Box-Cy55M-Award 2013

Safa Mellak

PhD Supervisor: Dr Stephane Potteaux

PARCC, INSERM U970, rue Leblanc 75015 Paris

Role of monocyte-derived macrophages (MØ**) and dendritic cells (DC) subsets in abdominal aortic aneurysm (AAA) formation and resolution**

AAA is widespread among elderly people and results in progressive expansion and rupture of the aorta with high mortality. AAA pathogenesis is characterized by an abnormal dilation of the aorta exceeding the normal diameter by 50% in response to multiple mechanisms including proteolysis, apoptosis and inflammation. The inflammatory process has long been known to contribute to the pathogenesis of AAAs and involves the infiltration of a variety of cells, leading to the over-regulation of multiple cytokines. The balance of the cellular type and resultant cytokine milieu determines the ultimate fate of the aortic wall healing, atherosclerosis or aneurysm formation.

Our aim is to determine the role of innate immunity in driving the cytokine milieu that characterizes this disease.

The murine model for AAA development used in our lab consists of implanting subcutaneous osmotic pumps delivering Angiotensin II (AngII) into C57BL6 ApoE-/- male mice. In order to assess the AngII induced inflammation at the vessel wall, we will use mice with an AT1 receptor ‚gain of function' mutant which is both constitutively active and hyperreactive to AngII.

The ImmunoTools IT-Box-Cy55M will help us perform in-vitro experiments on AT1 transgenic MØ and DC that we will differenciate from bone marrow monocytic populations using rm G-CSF or rm GM-CSF respectively, in order to characterize the innate immune responses in our AAA model.

Experimentally, AngII-stimulated MØ will be incubated with or without a well characterized combination of recombinant cytokines (rm IFNgamma, rm TNFα and

rm IL-1β) to reproduce inflammatory stimuli and screen the cytokine and chemokine panel produced by immunoassay of cultured medium.

Also, AngII-stimulated DC chemotaxis will be assessed by transwell-migration assays after incubation with a range of recombinant cytokines and chemokines rm IL-1β and rm TNFα, rm MIP3β / CCL19, rm IP-10 / CXCL10, rm MCP1 / CCL2). And last, we will perform coculture experiments with splenocytes (rmIL-2), in order to determine the immune polarization of helper T cells induced by AngII-stimulated DC.

These data will give us an insight into the AngII-related immuno-pathogenesis in AAA development.