SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa SIF estero

TIPOLOGIA DI RELAZIONE: Relazione fine periodo

TITOLO DELLA RELAZIONE: Defining the IL-21/IL-21 receptor axis role(s) in atherosclerosis

RELAZIONE:

Background

Cardiovascular diseases (CVD) are chronic inflammatory diseases characterized by a complex and constantly evolving tissue microenvironment (1). CVD is the underlying cause of about 30% of deaths worldwide, in this context, atherosclerosis represents the main factor of severe clinical manifestations such as myocardial infarction and ischemic stroke (2). Immunoinflammatory responses play a fundamental role in any stage of the atherosclerosis, from plaque formation to rupture (3, 4). Consequently, current research is focused on understanding what drives this inflammation and how it is regulated.
Interleukin 21 (IL-21) and its receptor (IL-21R) are key players in the immune response (5). Interleukin 21 (IL- 21) belongs to the IL-2 family of cytokines and is predominantly produced by T follicular helper (TFH), TH17 and NKT cells (5). The IL-21 receptor (IL-21R) is expressed by NK cells, T cells, B cells, dendritic cells and macrophages (5). The IL-21/IL-21R axis contributes to the pathogenesis of several autoimmune conditions characterized by an increased risk of vascular pathology. Interestingly, IL-21/IL-21R are also present in atherosclerotic vessels, but their role in this disease remains to be clarified.

Aim

This project aims to investigate the net contribution of the IL-21/IL-21R axis to atherosclerosis by using functional analysis, transcriptomics, mouse models of atherosclerosis, and access to human plaque cohorts. This study will provide important information on the regulation of the immune response in atherosclerosis, thus highlighting novel targets for specific immune therapies that could reduce cardiovascular morbidity and mortality.

Methods

Animals and experimental atherosclerosis

Experimental atherosclerosis has been induced at 8 weeks of age in WT and IL-21R$^{-/-}$ mice by gene transfer of proprotein convertase subtilisin/kexin type 9 (PCSK9) that binds hepatic low-density lipoprotein (LDL) receptors directing them for degradation in lysosomes. Briefly, a single intravenous AAV-PCSK9DY injection increases LDL levels in mice resulting in rapid and long-term sustained hyperlipidemia and atherosclerosis. This methodology allows testing of the genetic interaction of several mutations without the need for complex and time-consuming backcrosses with genetically modified hyperlipidaemic mice and is our model of choice. Following a single intravenous vector injection, mice were fed a high-fat Western diet (21% fat, 0.15% cholesterol, and 0% cholate). In the next few months, animals will be euthanized within 14 weeks after atherosclerosis induction and assessment of lesion area, fibrous cap, necrotic core, collagen content and calcification will be performed.

Analysis of atherosclerotic lesion

Atherosclerosis quantification will be performed using Oil red O (a marker of lipid accumulation) staining on lesions formed in the aortic sinus. Briefly, hearts will be perfused with ice-cold PBS; embedded in Tissue-Tec OCT (Tissue Tek, Sakura Finetek Europe, Zoeterwoude, The Netherlands), frozen at -80°C, and sectioned. Then, cryosections (10 µm) will be fixed in 10% neutral buffered formalin and rinsed with 60% isopropanol for 5 minutes, stained with 0.5% Oil Red O/60% isopropanol (20°C, 10 minutes), destained with 60% isopropanol for 2 minutes and extensively washed with distiller water. Finally, nuclei will be counterstained with hematoxylin. Images of stained sections will be acquired using an EVOS™ Imaging System (ThermoFisher Scientific) and the area occupied by the plaques in the sinus will be measured in a blinded fashion using the ImageJ software.

Collagen content

Aortic sinus sections will be stained with Picrosirius red and will be viewed with polarized light to detect collagen. For each animal, 10 sections will be analyzed, under blinded conditions, to determine the collagen content. The analyses will be carried out using Image J software and the results will be expressed as mean lesion area and mean percentage collagen area, respectively.

Calcification

Aortic sinus sections will be stained with Alizarin Red S for vascular calcification. After this, slides will be observed by microscopy (EVOS™ Imaging System; ThermoFisher Scientific). The analyses will be carried out using Image J software.

Fibrous Cap

Atherosclerotic plaque vulnerability is a vital clinical problem as vulnerable plaques tend to rupture, which results in atherosclerosis complications such as myocardial infarctions and subsequent cardiovascular deaths. Therefore, new strategies aiming to stabilize such plaques are in great demand. At the end of the experimental protocol we will characterize the fibrous cap thickness in both WT and IL-21$^{-/-}$ and assess plaque phenotype according to the Virmani's classification.

Conclusions

The experiments are currently in progress, therefore, in agreement with my supervisor, I have decided to extend my stay at the University of Glasgow, in order to complete the above-mentioned morphometric analyzes in both WT and IL-21$^{-/-}$ mice in the next 2-3 months. This will allow us to clarify whether the deficiency of the IL-21 receptor could limit atherosclerosis formation.

Finally, I would like to thank the Italian Society of Pharmacology (SIF) for the support it offers to young researchers, through which I had the opportunity to learn a broad range of new techniques,

be fully embedded in an international lab and attend courses on state-of-the-art technologies, thus increasing my professional portfolio of expertise.

References

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Data 11/04/2022

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