ACADEMIA ROMANA

INSTITUTUL DE BIOLOGIE SI PATOLOGIE CELULARA

"NICOLAE SIMIONESCU"

Manduteanu Ileana

MOLECULES AND MECHANISMS OF VASCULAR INFLAMMATION AS MOLECULAR TARGETS FOR THERAPIES

Habillitation Thesis

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To my mentors:

Professor Nicolae Simionescu and Professor Maya Simionescu

with deepest affection and gratitude

Summary

In the first section of my habilitation thesis, general data on inflammation in atherosclerosis, the state of the art and the personal contribution to the actual knowledge is presented. Based on our data and from the literature, we proposed (myself and my mentor), that although atherosclerosis is a continuous process, **a set of consecutive stages delineate atheroma development and fate** and provided an overview on the intersection of inflammation and dyslipidemia in atherosclerosis.

Next, **the novel - original contributions** on vascular cells dysfunction in inflammation associated with diabetes and atherosclerosis are presented. Our novel data on the mechanisms of the effects of high glucose (HG) and/or cytokines on endothelial cells (EC) activation revealed that: 1. HG induces valvular EC dysfunction; 2. HG, resistin (a cytokine) or HG in combination with resistin increases cell adhesion molecules and the subsequent monocyte adhesion by a process involving the chemokine fractalkine and P-selectin; 3. resistin and TNFα induce fractalkine expression at similar levels and do not have additive effects;. These findings, added novel data on the inflammatory potential of resistin and shedded new light on the cross-talk between resistin and TNF-α in human EC.

There is evidence that in humans, accelerated atherosclerosis occur in diabetes , and the intimal smooth muscle cells (SMC) switch to a secretory phenotype and are more pro-atherogenic by mechanisms not well defined . We investigated the role of HG and cytokine on SMC inflammation. Our **original** results demonstrated that HG induces overexpression of fractalkine and MCP-1 in SMC and subsequent increase in monocyte adhesion. In addition resistin induces a pro-inflammatory effect on SMC, increasing fractalkine and CX3CR1 expression by activating TLR4 and Gi proteins. Next, we questioned the mechanisms of interaction between SMC and monocytes within the plaque. Our novel data showed that as an outcome of the cross-talk between SMC and monocytes, several significant pro-inflammatory molecules are produced and the fractalkine /CXC3CR1 pair is specifically involved in TNF-a, MMP 9 and CX3CR1 upregulation. The cross-talk between SMC and monocytes activates JAK2/STAT 3 signaling pathway in both cells.

In an attempt to unravel new targets for imaging and therapy, I coordinated a complex project with a team of basic science and clinical researchers (cardiologists and rheumatologists). We revealed clinically relevant biomarkers and new targets for therapy and established the correlation between the human plasma levels of cytokines/chemokines and the stage of evolution of pathology from inflammation to either atherogenesis or the rheumatic disease.

As a part of the research direction on anti-inflammatory therapy, we unraveled new functions of ”old” drugs (enoxaparin and aspirin). We discovered a novel attribute of enoxaparin that is the inhibition of monocyte adhesion to EC activated by high glucose or cytokines by mechanisms involving cell adhesion molecules. Aspirin, PPARs agonists fenofibrate and clofibrate inhibit the HG-induced increased fractalkine and MCP-1 expression in human EC by specific mechanisms. In addition we demonstrated that the natural anti-oxidants, curcumin and Morus Alba extracts reduce resistin-activated EC.

Another approach to anti-inflammatory therapy is the use of nanomedicine. We have prepared “intelligent” liposomal systems (recognizing EC-VCAM-1), loaded with drugs and able to target activated EC covering the lesional areas in atherosclerosis.

The last part of Section 1 includes the **Professional Results** where I give details on the dissemination of my main scientific results**,** obtained after my phD degree: 24 original articles indexed ISI, with 580 citations, 6 non-ISI articles, 3 book chapters , 57 communications at international-, and 43 at national scientific meetings; my H index is 11. I was recognized by 3 scientific awards, one national and 2 international.

My **Management Activity** consisted in participation to national and international projects, in 5 as director and 8 as collaborator, and in one international grant as part of the management and WP leader. Then I present my **other activities** comprising participation as official referee or president of PhD commissions and in competitions for appointments, invited reviewer for various international journals, expert for national grants, and I worked as an expert in CNATDCU commission. At present, I am head of the Department of Biopathology and Therapy of Inflammation, and as deputy director of ICBP. My activity is focused on coordinating my team of researchers, helping them to implement their projects at maximal performance, to help young people to permanently improve their knowledge. Another preoccupation is to improve our collaborations with clinicians and also with groups from other countries working in our area of research.

In the Section II, it is detailed the direction of the present research project. Our goals are to search for the mechanisms by which mediators modulate the inflammation in EC, SMC, monocytes /macrophages, and get insights in the signalling mechanisms and gene regulation involved, and develop new targeted nanotechnology-based therapeutic strategies to reduce

vascular wall inflammation. We believe that the data will be applicable to the numerous diseases in which inflammation is involved.

At the end of Section II, I present the submitted projects, my future plans of evolution of the carier thinking on performing mentorat at “Simionescu”’s standards.

I am continuing my endeavor, thinking to what Professor Nicolae Simionescu said: « Nothing durable can be done if in the construction material is not added « quatum satis », soul. Since we know that “without giving, you can never become what you admire” we are lucky to have as a model Dr. Maya Simionescu that continues to trust and to make us believe that « the impossible does not exist ».