Biomedical Studies

Teresa Pinheiro

The research activities during 2006 within the Biomedical Studies group made use of focused ion beam techniques to image tissue and cell morphology, as well as other microscopy techniques and methodological procedures to assess molecular indicators of cell/tissue response, e.g. using flux citometry technique, molecular biology and biochemistry assays.

The outcome of past and ongoing research projects in the context of atherosclerosis paved the way to the present main activities. Since 1991, several projects gathering different teams of the Faculty of Sciences and ITN among others, allowed to carry out a number of human health studies involving Portuguese populations in health and disease conditions. Examples of these applications are the study of skin permeability to nano--particles dispersed in cosmetics, skin as a surrogate marker in metabolic diseases (e.g. haemochromatosis), oxidative stress and inflammation markers for chronic pulmonary diseases, and for atherosclerosis. Beyond their strict scientific interest, they also revealed to be important in terms of Public Health for the studied populations and allowed comparative analyses of data from different regions.

The studies on dermatology and haematology, combining indicators of the inflammatory response and metabolism, are naturally continuing as confirmed by renewed financial support of private and international entities.

The recent and significant funding of several projects in association with the Serviço de Cardiologia, Hospital de Santa Marta, Lisboa, stimulated the research activities to develop a clinical registry of inflammation in acute coronary syndromes and to monitor the alterations of that process during the recovery period. The expected results will have important impact on the understanding of the inflammatory process and cell signalling, and consequently may be useful in prevention and treatment of acute myocardial infarction. These issues are being recognized by the scientific community, as attested by the increasing number of publications in high impact journals.

The work performed is carried out exclusively under research contracts that associate several national and international research centres. The multidisciplinary characteristic of the joint teams also permit to attract young scientists.

Apart from research activities, technical services are provided to private institutions, mainly characterisation of raw materials for the pharmaceutical industry.

The main achievements of the research developed during 2006 are summarised in the following pages.

Researchers(*)
T. PINHEIRO, Aux. Researcher
L.C. ALVES, Aux. Researcher

Students
P. NAPOLEÃO, Ph.D. student, FCT grant

Technical Personnel
R. PINHEIRO, laboratory assistant

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Inflammation in the evolution of acute myocardial infarction and cardiac failure

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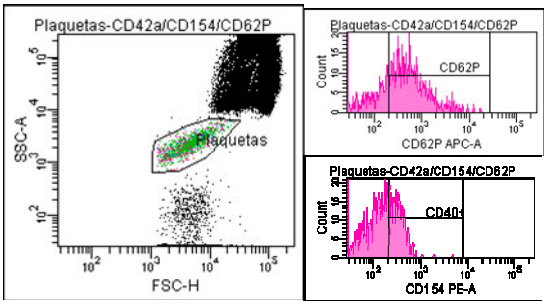
Inflammation and endothelial injury are now considered key components of atherosclerosis from fatty streak formation to plaque rupture, subsequent thrombosis, and progressive mechanical and dynamic obstruction. Rupture of the fibrous cap of arterial plaque, exposes tissue factors present in the necrotic core, triggering inflammatory signalling, cell adhesion, and coagulation cascade that eventually leads to thrombus.

Cell activation plays a predominant role in the progression of atherosclerosis as microparticles are produced by blebbing of the activated cell and their release in circulation may irreversibly amplify inflammation. In this process pro-inflammatory cytokines play a significant role, since they elicit the expression of adhesion molecules and other cytokines, which induces expression of acute-phase reactants, and thus contribute to a state of chronic inflammation.

Based on this outcome the major objective of the current projects is to investigate the microparticle production by the endothelium, platelets and lymphocytes and to identify the expression of inflammatory molecules in the cells that originate them in cardiovascular diseases. Also, soluble forms of indicators of the inflammatory response are assessed in circulation. Blood measurements of nitric oxide, iron, and oxidised LDL, of the cytokine TNF-a, soluble CD40L and soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin, are some of the parameters being assessed. Data is being linked to risk factors, co-morbidity indicators and therapy. Measurements are carried out using multiple approaches from immuno-assays supported by spectrophotometry to flux citometry. The groups of study consist of patients with acute coronary syndrome (MI), cardiac failure (IC), which are compared with a reference group of healthy volunteers and/or a group control for coronaries. A longitudinal follow-up of MI group is being carried out at three different occasions: in the first 24 hours of evolution of the acute myocardial infarction (considered as day 0); two days after (day 2); and approximately two months after (day 40). In cardiac failure, patients are assessed before intervention and after intervention at the first and sixth month of recovery.

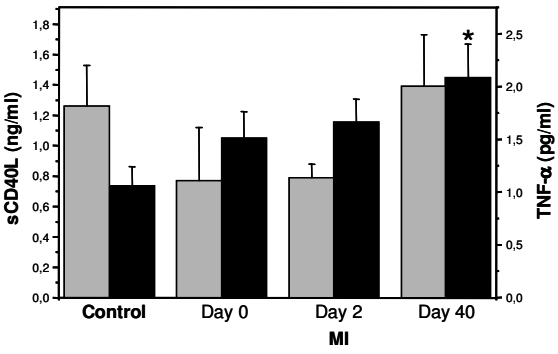
Variations in the number of microparticles were observed as well as in inflammatory markers measured, emphasizing the importance of systemic inflammation in the disease evaluation. The increased concentration and expression of sP-selectin at the infarct onset evidenced the functional state of thrombocytes and the endothelial cells that play an important role in the rupture or erosion of atherosclerotic plaque (Fig. 1).

Fig. 1: Expression of P-selectin (CD62P) and CD40L (CD154) in platelets assessed by flux citometry.



Later on during recovering period when haemodynamic variables lean to stability in part due to synergistic action of physiological and pharmacological effects, the increase in circulating sCD40L levels and cytokine levels as TNF-α (Fig. 2) may express the role of these molecules in the endothelial and myocardial tissues recuperation.

Fig. 2: Concentrations of soluble CD40L (gray) and TNF-α (black) in blood of control and MI groups (*significantly different from control group, $p<0.05$).



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Using skin as a diagnosis tool in hemochromatosis and psoriasis

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1 - The causes of hemochromatosis include defects in genes encoding HFE, ferroportin, hepcidin, among others. The liver dysfunction in hepcidin synthesis has been associated to most known forms of hemochromatosis. The present study aim at diagnosing hemochromatosis before irreversible damage develops. The study used conventional and innovative laboratory tests to differentiate distortions of iron metabolism. So far, 24 patients were genetically characterized and studied before starting and along the phlebotomy therapy. Nuclear microscopy and nuclear resonance techniques provided iron quantitative imaging and physiological information or skin and liver. Biochemical methods provided hepcidin contents in serum and markers of iron metabolism and organ function e.g., ferritin, transferrin saturation, transferase activity, glucose, etc. Before starting and at initial phases of therapy high concentration levels of Fe in skin, liver and plasma were observed, followed by a sharp decrease in all tissues as therapy advances. These variations are all correlated. When therapy is interrupted there is a trend of Fe increase in plasma/serum. Hepcidin concentration in serum does not discriminate between controls and patients but associates inversely with skin Fe. This suggests that iron loading in hemochromatosis is not due to inappropriate hepcidin concentration in serum. The associations between iron concentrations in skin and liver and circulating contents of hepcidin in patients before starting and along treatment will help to a better understanding of iron pools mobilisation. The study is supported by the projects SPDV 2004-2007 and IAEA CRP 2005-2007.

2 - Psoriasis is a skin disorder characterised by an increased proliferation and disturbed differentiation of keratinocytes. Current therapies, photo(chemo)therapy and systemic biological agents for moderate to severe psoriasis can control the disease but are not curative. The main objective of the study is to characterise the immune pattern and the markers of the inflammatory cascade, hyperproliferation and keratinisation in the psoriatic lesions of patients with moderate to severe plaque psoriasis. The individual response to conventional PUVA and narrow band UVB therapies and systemic immuno-target drugs, such as, etanercept and efaluzimab will be assessed through Psoriasis Area and Severity Index (PASI) and histological indexes based on keratinisation and epidermis hyperplasia reduction. T-lymphocyte profile in responders and non-responders, their activation profile and how it correlates with TNF-a and keratinocyte hyperproliferation and keratinisation, the skin barrier function and the involvement of calcium and other divalent ions distribution in skin strata, are some of the aspects that are being covered in the project. This Project is supported by SERONO, Fundación Salud 2000 (Research Prize "Investigação Clínica em Psoríase" - 2006-2008).

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Skin permeability to nanoparticles

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Nanoparticles of TiO_2 and ZnO are widely used in commercial sunscreens by their capacity to scatter UV wavelengths of sunlight. Skin exposure to commercial products containing nanoparticles of Ti, Zn, and Si oxides, among others and their trans-epidermal diffusion has been studied using nuclear microscopy techniques. One of the major achievements of the work was establishing the percutaneous penetration depth of Ti oxides and ZnO. Therefore, methodologies were adjusted to enable the validation of elemental distribution maps or profiles with high-resolution images originated in transmission mode (STIM, Scanning Transmission Ion Microscopy). The work has been carried out under a consortium EC/QLK4-CT-2002-02678.

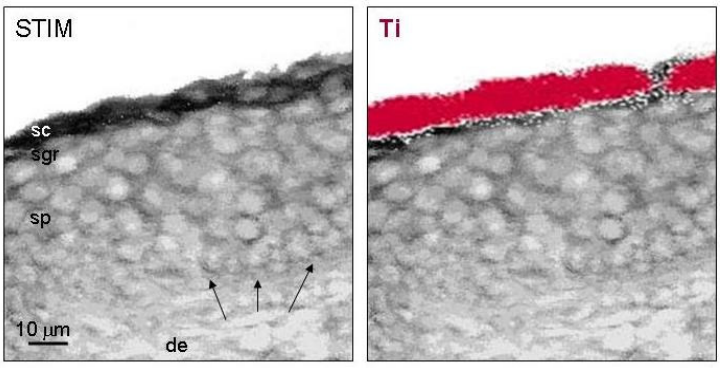

Fig.1: High-resolution images of skin (STIM). Each cell layer can be identified enabling the accurate determination of the penetration depth of nanoparticles. sc- str. corneum; sgr - str. granulosum; sp - str. spinosum; de - dermis; arrows - str. germinativum.

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