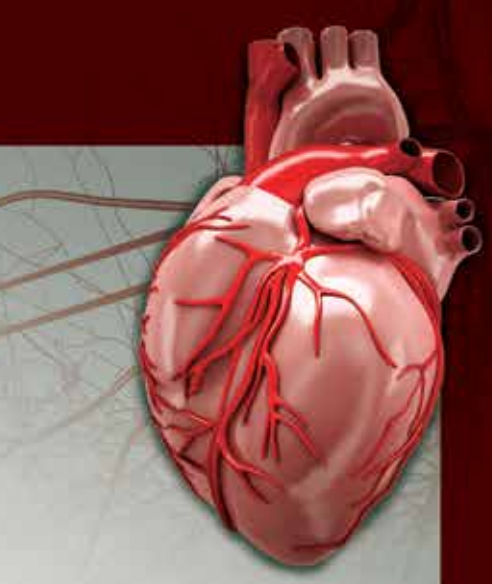
Inflammation in cardiovascular disease



Dr Giuseppina Caligiuri explains the role of CD31 in atherosclerosis and describes her research into the inflammatory immune response that contributes to cardiovascular disease

Can you summarise the main objectives of your research? How has this evolved over the 20 years that you have been working on atherosclerosis?

My research focuses on the inflammatory-immune components of atherosclerosis – the hardening and narrowing of arteries. As an undergraduate medical student I had the chance to perform my clinical training in Rome, Italy, under the supervision of Professor Attilio Maseri, who noticed that patients with acute coronary syndromes display systemic signs of inflammation. I analysed the inflammatory cells present in patients' blood and found that T cells, involved in specific immune responses, were particularly activated in patients with the most severe degree of the disease.

Following this, my curiosity was piqued and I decided to pursue a PhD programme at the Karolinska Institute in Stockholm, Sweden, under the supervision of Professor Göran Hanson – an expert in the field of immune response and atherosclerosis. There, I learnt how complex the immune system is and gathered experimental evidence in favour of the existence of both harmful and protective immune components.

What is the normal function of cluster of differentiation 31 (CD31) and the purpose of its expression by the cells of the immune system?

Among the immune-regulatory receptors, we have been particularly interested in exploring the function of the CD31 protein because it is self-binding and constitutively and exclusively expressed by the immune cells that interact with the cells of the blood-vessel interface. CD31-mediated cell interaction drives a detachment signal – allowing the free flow of immune cells and platelets suspended in the blood by raising their activation threshold; maintaining them in a resting state unless a strong noxious stimulus accesses the system. Interestingly, the frequency of T cells with cleaved non-functional CD31 in the blood of patients with acute coronary syndromes, and experimental atherosclerotic mice, is significantly higher than in controls. This suggests that occurrence of the immune-mediated complications of atherosclerotic arteries is linked to the lack of CD31 on T cells.

How much progress have you made since this finding towards developing a therapeutic strategy for atherosclerosis and other inflammatory diseases?

CD31 is a very attractive and novel therapeutic target because it is lost by activated cells of the immune system – as well as by platelets and endothelial cells – and the beneficial effects of a specific treatment can restore its regulatory function and control the activation of these cells. With this in mind, we have developed a specific test to measure the extent of CD31 cleavage from different cell types. This tool is useful for diagnostic/prognostic purposes and for guiding the dosage of treatments in patients throughout the waxing and waning phases of inflammation.

In parallel, we have also developed a candidate CD31-specific therapeutic drug able to sustain and rescue the regulatory functions of CD31, even in those cells where it has been cleaved. Interestingly, preliminary data suggest beneficial roles, not only in atherosclerosis, but also in other chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, as well as in allograft rejection.

Furthermore, we have also conceived a local use of the drug if coated on endovascular prostheses, such as arterial stents and flow diverters, with preliminary data suggesting that the devices coated with this peptide are more biocompatible and less prone to drive complications.

How does your research fit into the wider aims of the Laboratory for Vascular Translational Science?

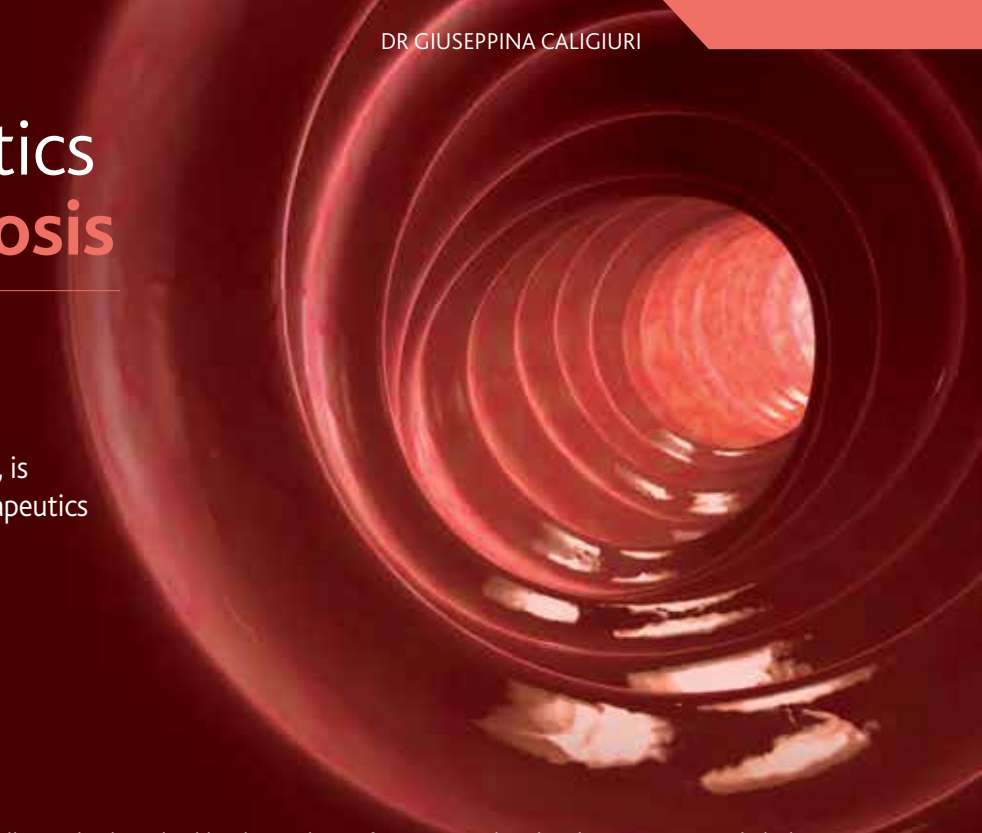
The Laboratory for Vascular Translational Science is the ideal research environment for me. Here, I can interact on a daily basis with unique scientists, such as Professors Laurent Feldman and Phillipe Gabriel Steg, both recognised as experts in clinical and translational research in acute coronary syndromes; and Dr Jean-Baptiste Michel, an internationally recognised expert in both vascular cell biology and *in vivo* experimental models of vascular pathology. Furthermore, there is Professor Antonino Nicoletti, leader of the group I belong to and expert in fundamental immunology; and Dr Didier Letourneur, a leader in bioengineering using natural molecules. All these scientists contribute to the success of my work. The rich discussions that I can engage in with these, and many other colleagues, are fundamental for the type of research I love to do.

Are you collaborating with any external laboratories or organisations?

The translational research I perform requires several different and complementary types of expertise and technical approaches. The collaborations and co-developmental programmes we have established with industrial leaders are essential for the development of our strategies for clinical use. A wide gap exists between academic and industrial science so initiatives aimed at promoting interaction between these different areas and developers would be extremely beneficial for medical science.

DR GIUSEPPINA CALIGIURI

Novel therapeutics for atherosclerosis



The life-threatening complications of atherosclerotic arteries are linked to an uncontrolled immune-inflammatory response. Research being carried out at **Inserm** within the Bichat Hospital, France, is developing novel immunoregulatory therapeutics that promise to revolutionise treatment

CARDIOVASCULAR DISEASE (CVD) is responsible for 17 million deaths each year, making it the leading global cause of mortality. A major risk factor for CVD is atherosclerosis – the hardening of arteries due to the formation of an atheromatous plaque of fatty lipids and collagen. The small arteries in the heart or brain become narrowed by fatty deposits and can suddenly be occluded by a blood clot, causing heart attacks or strokes respectively.

Blood-derived cholesterol accumulates in the sub-endothelial space, which signals to the immune system that a toxic substance is present. The immune cells promote an inflammatory response in the vascular wall to engulf the lipids and promote the formation of a fibrous cap of collagen, aiming to prevent the lipidic core from forming a blood clot. However, the continued accumulation of cholesterol and inflammation at the artery wall causes the plaque to grow, narrowing the lumen, and eventually bursting to release a clot that can suddenly block the artery.

Dr Giuseppina Caligiuri is an associated cardiologist fellow at Bichat Hospital and Research Director at Inserm U1148 (LVTS), where she is the co-leader of the Immunopathology and Immunomodulation of Cardiovascular Diseases group. Her work on the role of inflammation and immune responses in atherosclerosis has led to a greater understanding of the beneficial and harmful elements involved.

Inflammation is a typical immune system response to harmful stimuli that is closely regulated by the body. Caligiuri's early work showed that certain antibody-producing B cells and reparative macrophages slow the development of the disease while other facets of the immune system have a pathogenic role. "The target of the pathogenic immune response is present in both atherosclerotic patients and healthy subjects, but the former have a lower threshold for T cell activation and a higher affinity of autoreactive antibodies," explains Caligiuri.

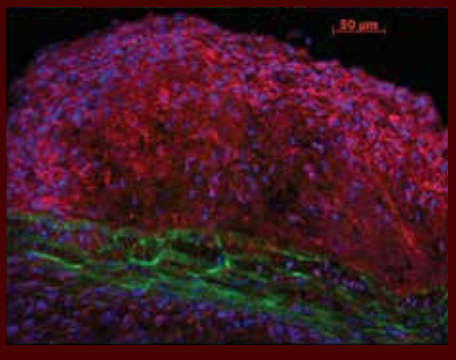
CD31 LOSS PROMOTES ATHEROSCLEROSIS

Caligiuri's research focuses on a regulatory receptor called cluster of differentiation 31 (CD31), which is present on the surface of cells involved in the blood vessel interface – specifically T cells, other white blood cells, platelets and the endothelial surface of arteries. CD31 is particularly interesting because it binds other molecules of its kind on cells with which it interacts, allowing them to detach from each other, and from the artery wall – promoting the smooth flow of blood through the vessel. The number of T cells coated with CD31 decreases with age, a finding that caused Caligiuri to question whether this loss of regulation leads to defective immune tolerance in the vascular bed and the formation/progression of atherosclerotic plaques.

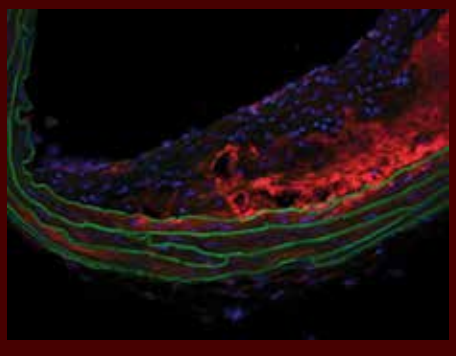
Using a mouse model prone to atherosclerosis – the apolipoprotein E knockout mouse – Caligiuri found that individuals with severe atherosclerotic disease had significantly fewer CD31-positive T cells in their blood and, importantly, T cells within the plaques were mostly lacking CD31. A similar effect was seen in human patients with clinical manifestations of atherosclerosis. Caligiuri also used this mouse model to overexpress CD31, resulting in reduced T cell penetration of the artery endothelium and the prevention of plaque development. Taken together, these results indicate that the interaction of T cells lacking CD31 with the artery endothelium cannot be properly regulated and, consequently, are more easily activated to invade cholesterol-filled endothelium and facilitate the abnormal inflammatory response of atherosclerosis.

DIAGNOSTIC TOOL DEVELOPMENT

CD31 is a single chain molecule containing six functional domains outside the cell, a membrane-spanning region and a long tail extending into the cell's cytoplasm. The intracellular region has



Confocal microscopy micrograph of an experimental atherosclerotic plaque from an apolipoprotein E knockout mouse. Fluorescent immunostaining shows that most of the cells composing the plaque, indicated by the presence of nuclei (DAPI, blue) are macrophages (Mac3 staining, red) and that the extracellular matrix degrading metalloproteinase 9 enzyme (MMP9, purple) is widespread within the plaque. The elastin fibres (green) appear degraded.



Treatment with a CD31 agonist in the same experimental model of atherosclerosis was able to reduce the degradation of the extracellular matrix (the elastin fibres are parallel and consistent) and inflammatory component of atherosclerotic plaques (most of the cells, indicated by the nuclei, are not macrophages). The red staining corresponds to the necrotic core, which is hidden by non-inflammatory cells.

INTELLIGENCE

ROLE OF IMMUNE-INFLAMMATORY RESPONSES IN ATHEROSCLEROSIS

OBJECTIVES

To identify the putative triggers underlying the characteristic inflammatory state of patients at risk of cardiovascular events.

KEY COLLABORATORS

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Drs Peter and **Debra Newman**, Milwaukee Blood Center of Wisconsin, USA

Dr Frans Nauwelaers, BD Biosciences, Belgium

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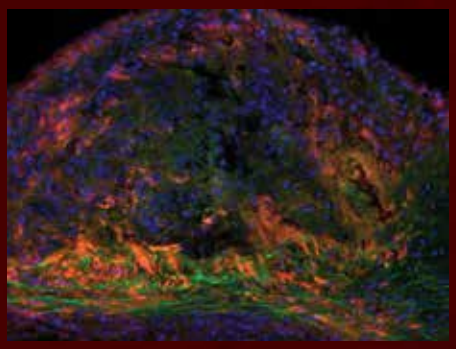
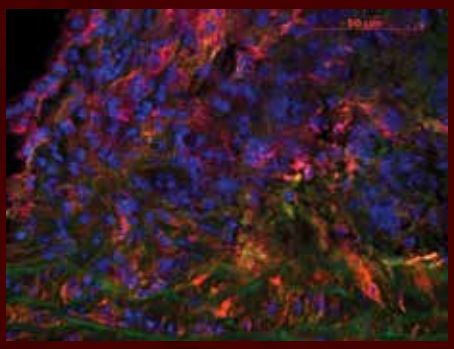
GIUSEPPINA CALIGIURI was awarded her MD in 1993 from the Catholic University in Rome, Italy, before going on to complete a postgraduate specialisation in cardiology. She then studied at the Karolinska Institute, Sweden, and the University of Paris, France. She is currently Research Director at Inserm, and has published 90 peer-reviewed articles.





Left: Fluorescent staining of VCAM (an adhesion and pro-inflammatory molecule, red) in a microscopic section of an experimental atherosclerotic plaque (apolipoprotein E knockout mice), and of alpha smooth muscle actin (green). This shows that, in addition to the inflammatory cells composing the plaque, the smooth muscle cells of the arterial wall have acquired an inflammatory phenotype (yellow indicates positive staining for both VCAM, red and alpha smooth muscle actin, green). **Right**: Detail at higher magnification of the left-hand image, showing the destruction of the arterial wall – degradation of the elastic fibres and dense infiltration of the plaque and arterial wall by VCAM+ inflammatory cells.

two immunoreceptor tyrosine-based inhibition motifs that exert the negative signalling function involved in maintaining CD31-positive cells in a resting state.

Caligiuri showed that CD31-negative cells are not completely lacking the entire CD31; the molecule is cleaved by activated cells, releasing much of the extracellular section but leaving the cytoplasmic tail intact. Cleaved CD31 is no longer able to bind with CD31 molecules of other cells, resulting in a loss of its regulatory function. The truncated portion released from activated cells vary in length, according to the cell of origin (leukocyte, platelet, endothelial cell), and can be specifically detected as a freely soluble protein in human blood plasma.

Caligiuri has begun developing a laboratory serum CD31 test for clinical use as a diagnostic and monitoring tool. Cleaved and complete CD31 molecules are removed from blood plasma and treated with antibodies against three of the six extracellular domains, as well as the cytoplasmic tail. By analysing the quantity of each antibody-bound domain it is possible to determine the relative amounts of cleaved and whole CD31 molecules in the blood. Cleaved CD31 levels are much higher than normal in atherosclerotic patients, allowing rapid and efficient clinical diagnosis, facilitating decision making about treatment type, prognosis estimation and drug efficacy monitoring.

RESTORING THE REGULATORY FUNCTIONS OF CLEAVED CD31

Upon CD31 cleavage, a small fragment of the receptor is left behind on the outer surface of the cell. Caligiuri has discovered that this is able to bind a complementary synthetic CD31 peptide, an association that is sufficient to inhibit T cell activation to the extent that CD31-negative T cells become stable and regulatory. "The physicochemical, toxicologic and pharmachodynamic properties of the candidate drug are favourable," states Caligiuri, who is currently in negotiations with the pharmaceutical industry to develop the CD31 agonist peptide for clinical use. Her work using experimental mouse models has shown that the drug could be beneficial, not only for preventing/repairing atherosclerotic complications, but also for systemic treatment of many chronic inflammatory conditions, including arthritis and multiple sclerosis.

BIOACTIVE STENTS PREVENT SIDE-EFFECTS

When coronary arteries become atherosclerotic, an angioplasty procedure can be used to reopen the vessel and a wire mesh tube called a stent is often implanted to keep the vessel patent. Unfortunately, because the stent is recognised as a foreign body, affected arteries can become the site of a chronic inflammatory process that leads to restenosis – a recurrence of arterial narrowing. This can be prevented, to a certain extent, using a drug-eluting stent. Unfortunately, this solution prevents the endothelial cells from covering the metal struts, meaning that new blood clots continue to be a problem for these prostheses, and anticoagulant drugs must be taken for at least a year after surgery.

In an attempt to solve the problems associated with the use of arterial stents, Caligiuri and her collaborators have developed an innovative stent coated with their CD31-binding synthetic peptide to provide anticlotting and anti-inflammatory properties, as well as promoting the growth of the endothelial cells. This provides reparative qualities to the implant and demonstrates that the CD31 agonist peptide represents a significant step towards eliminating the stent-associated complications.

PAVING THE WAY FOR TREATMENTS

Caligiuri believes her novel therapeutics could lead to a whole new style of treatment for atherosclerosis and other chronic inflammatory conditions. "Currently, most of the immunotherapeutic arsenal consists of molecules that block one signalling pathway. This approach is plagued with inherent drug-resistance that arises from the redundancy of inflammatory pathways and inevitable unwanted side-effects due to the depletion of a given immune cell type," she explains. Caligiuri hopes that the CD31-targeting drug will be the first of a novel class of 'immunoregulatory' rather than 'immunosuppressive' therapies that are effective with lower associated risks of infection and disease recurrence.