Année de publication : 2020

David M Smadja, Richard Chocron, Elisa Rossi, Bastien Poitier, Yuri Pya, Mahabbat Bekbossynova, Christophe Peronino, Jeanne Rancic, Jean Christian Roussel, Michel Kindo, Nicolas Gendron, Ludovica Migliozzi, Antoine Capel, Jean Christophe Perles, Pascale Gaussem, Peter Ivak, Piet Jansen, Claude Girard, Alain Carpentier, Christian Latremouille, Coralie Guerin, Ivan Netuka (2020 Jul 21)

Autoregulation of Pulsatile Bioprosthetic Total Artificial Heart is Involved in Endothelial Homeostasis Preservation.

Thrombosis and haemostasis : DOI : 10.1055/s-0040-1713751

Résumé

Pulsatile Carmat bioprosthetic total artificial heart (C-TAH) is designed to be implanted in patients with biventricular end-stage heart failure. Since flow variation might contribute to endothelial dysfunction, we explored circulating endothelial biomarkers after C-TAH implantation in seven patients and compared the manual and autoregulated mode. Markers of endothelial dysfunction and regeneration were compared before and during a 6- to 9-month follow-up after implantation. The follow-up was divided into three periods (< 3, 3-6, and > 6 months) and used to estimate the temporal trends during the study period. A linear mixed model was used to analyze repeated measures and association between tested parameters according to the mode of C-TAH and the time. Relevance of soluble endoglin (sEndoglin) level increase has been tested on differentiation and migration potential of human vasculogenic progenitor cells (endothelial colony forming cells [ECFCs]). Normal sEndoglin and soluble endothelial protein C receptor (sEPCR) levels were found in patients after implantation with autoregulated C-TAH, whereas they significantly increased in the manual mode, as compared with pretransplant values (= 0.005 and 0.001, respectively). In the autoregulated mode, a significant increase in the mobilization of cytokine stromal cell-derived factor 1 was found (= 0.03). After adjustment on the mode of C-TAH, creatinine or C-reactive protein level, sEndoglin, and sEPCR, were found significantly associated with plasma total protein levels. Moreover, a significant decrease in pseudotubes formation and migration ability was observed in vitro in ECFCs receiving sEndoglin activation. Our combined analysis of endothelial biomarkers confirms the favorable impact of blood flow variation achieved with autoregulation in patients implanted with the bioprosthetic total artificial heart.

Léa Guyonnet, Alicja Czopek, Tariq E Farrah, Véronique Baudrie, Philippe Bonnin, Anna Chipont, Olivia Lenoir, Florian Sennlaub, Christophe Roubeix, David J Webb, David C Kluth, Matthew A Bailey, Pierre-Louis Tharaux, Neeraj Dhaun (2020 Jun 23)

Deletion of the myeloid endothelin-B receptor confers long-term protection from angiotensin II-mediated kidney, eye and vessel injury.

Kidney international : DOI : S0085-2538(20)30690-6

Résumé

The endothelin system may be an important player in hypertensive end-organ injury as endothelin-1 increases blood pressure and is pro-inflammatory. The immune system is emerging as an important regulator of blood pressure and we have shown that the early hypertensive response to angiotensin-II infusion was amplified in mice deficient of myeloid endothelin-B (ET) receptors (LysM-CreEdnrblox/lox). Hypothesizing that these mice would display enhanced organ injury, we gave angiotensin-II to LysM-CreEdnrblox/lox and littermate controls (Ednrblox/lox) for six weeks. Unexpectedly, LysM-CreEdnrblox/lox mice were significantly protected from organ injury, with less proteinuria, glomerulosclerosis and inflammation of the kidney compared to controls. In the eye, LysM-CreEdnrblox/lox mice had fewer retinal hemorrhages, less microglial activation and less vessel rarefaction. Cardiac remodeling and dysfunction were similar in both groups at week six but LysM-CreEdnrblox/lox mice had better endothelial function. Although blood pressure was initially higher in LysM-CreEdnrblox/lox mice, this was not sustained. A natriuretic switch at about two weeks, due to enhanced ET signaling in the kidney, induced a hypertensive reversal. By week six, blood pressure was lower in LysM-CreEdnrblox/lox mice than in controls. At six weeks, macrophages from LysM-CreEdnrblox/lox mice were more anti-inflammatory and had greater phagocytic ability compared to the macrophages of Ednrblox/lox mice. Thus, myeloid cell ET receptor signaling drives this injury both through amplifying hypertension and by inflammatory polarization of macrophages.

Jean-Luc Diehl, Jean Loup Augy, Nadia Rivet, Coralie Guerin, Richard Chocron, David M Smadja (2020 Jun 11)

Severity of endothelial dysfunction is associated with the occurrence of hemorrhagic complications in COPD patients treated by extracorporeal CO removal.

Intensive care medicine : DOI : 10.1007/s00134-020-06138-8

Résumé

David M Smadja, Coralie L Guerin, Richard Chocron, Nader Yatim, Jeremy Boussier, Nicolas Gendron, Lina Khider, Jérôme Hadjadj, Guillaume Goudot, Benjamin Debuc, Philippe Juvin, Caroline Hauw-Berlemont, Jean-Loup Augy, Nicolas Peron, Emmanuel Messas, Benjamin Planquette, Olivier Sanchez, Bruno Charbit, Pascale Gaussem, Darragh Duffy, Benjamin Terrier, Tristan Mirault, Jean-Luc Diehl (2020 May 28)

Angiopoietin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID-19 patients.

Angiogenesis : DOI : 10.1007/s10456-020-09730-0

Résumé

Coronavirus disease-2019 (COVID-19), a respiratory disease has been associated with ischemic complications, coagulation disorders, and an endotheliitis.

Nathalie Nevo, Severine Lecourt, Ivan Bièche, Magda Kucia, Audrey Cras, Adeline Blandinieres, Sophie Vacher, Nicolas Gendron, Coralie L Guerin, Mariusz Z Ratajczak, David M Smadja (2020 Jan 4)

Valproic Acid Decreases Endothelial Colony Forming Cells Differentiation and Induces Endothelial-to-Mesenchymal Transition-like Process.

Stem cell reviews and reports : 357-368 : DOI : 10.1007/s12015-019-09950-y

Résumé

Valproic acid (VPA), a histone deacetylase (HDAC) inhibitor is a widely used anticonvulsant drug. VPA is also under clinical evaluation to be employed in anticancer therapy, as an antithrombotic agent or a molecule to be used in the stem cells expansion protocols. Since endothelial colony forming cells (ECFC) has been identified as the human postnatal vasculogenic cells involved in thrombotic disorders and serve as a promising source of immature cell for vascular repair, objectives of the present study were to determine how VPA contributes to ECFC commitment and their angiogenic properties. We examined the effect of VPA on ECFC obtained from cord blood by evaluating colony number, proliferation, migration and their sprouting ability in vitro, as well as their in vivo vasculogenic properties. VPA inhibited endothelial differentiation potential from of cord blood derived stem cells associated with decreased proliferation and sprouting activity of cultured ECFC. VPA treatment significantly decreased the vessel-forming ability of ECFC transplanted together with mesenchymal stem cells (MSC) in Matrigel implants in nude mice model. Surprisingly, a microscopic evaluation revealed that VPA induces marked morphological changes from a cobblestone-like EC morphology to enlarged spindle shaped morphology of ECFC. RT-qPCR and a CD31/CD90 flow cytometry analysis confirmed a phenotypic switch of VPA-treated ECFC to mesenchymal-like phenotype. In conclusion, the pan-HDAC inhibitor VPA described for expansion of hematopoietic stem cells and very small embryonic like stem cells cannot be successfully employed for differentiation of endothelial lineage committed ECFC into functional endothelial cells. Our data also suggest that VPA based therapeutics may induce endothelial dysfunction associated with fibrosis that might induce thrombosis recurrence or venous insufficiency.

Année de publication : 2019

Ulysse Richez, Hector De Castilla, Coralie L Guerin, Nicolas Gendron, Giulia Luraghi, Marc Grimme, Wei Wu, Myriam Taverna, Piet Jansen, Christian Latremouille, Francesco Migliavacca, Gabriele Dubini, Antoine Capel, Alain Carpentier, David M Smadja (2019 Dec 24)

Hemocompatibility and safety of the Carmat Total Artifical Heart hybrid membrane.

Heliyon : e02914 : DOI : 10.1016/j.heliyon.2019.e02914

Résumé

The Carmat bioprosthetic total artificial heart (C-TAH) is a biventricular pump developed to minimize drawbacks of current mechanical assist devices and improve quality of life during

support. This study aims to evaluate the safety of the hybrid membrane, which plays a pivotal role in this artificial heart. We investigated in particular its blood-contacting surface layer of bovine pericardial tissue, in terms of mechanical aging, risks of calcification, and impact of the hemodynamics shear stress inside the ventricles on blood components. Hybrid membranes were aged in a custom-designed endurance bench. Mechanical, physical and chemical properties were not significantly modified from 9 months up to 4 years of aging using a simulating process. Exploration of erosion areas did not show no risk of oil diffusion through the membrane. Blood contacting materials in the ventricular cavities were subcutaneously implanted in Wistar rats for 30 days as a model for calcification and demonstrated that the in-house anti-calcification pretreatment with Formaldehyde-Ethanol-Tween 80 was able to significantly reduce the calcium concentration from 132 µg/mg to 4.42 µg/mg ($p < 0.001$). Hemodynamic simulations with a computational model were used to reproduce shear stress in left and right ventricles and no significant stress was able to trigger hemolysis, platelet activation nor degradation of the von Willebrand factor multimers. Moreover, explanted hybrid membranes from patients included in the feasibility clinical study were analyzed confirming preclinical results with the absence of significant membrane calcification. At last, blood plasma bank analysis from the four patients implanted with C-TAH during the feasibility study showed no residual glutaraldehyde increase in plasma and confirmed hemodynamic simulation-based results with the absence of hemolysis and platelet activation associated with normal levels of plasma free hemoglobin and platelet microparticles after C-TAH implantation. These results on mechanical aging, calcification model and hemodynamic simulations predicted the safety of the hybrid membrane used in the C-TAH, and were confirmed in the feasibility study.

Hélène Lazareth, Carole Henique, Olivia Lenoir, Victor G Puelles, Martin Flamant, Guillaume Bollée, Cécile Fligny, Marine Camus, Lea Guyonnet, Corinne Millien, François Gaillard, Anna Chipont, Blaise Robin, Sylvie Fabrega, Neeraj Dhaun, Eric Camerer, Oliver Kretz, Florian Grahammer, Fabian Braun, Tobias B Huber, Dominique Nochy, Chantal Mandet, Patrick Bruneval, Laurent Mesnard, Eric Thervet, Alexandre Karras, François Le Naour, Eric Rubinstein, Claude Boucheix, Antigoni Alexandrou, Marcus J Moeller, Cédric Bouzigues, Pierre-Louis Tharaux (2019 Jul 26)

The tetraspanin CD9 controls migration and proliferation of parietal epithelial cells and glomerular disease progression.

Nature communications : 3303 : DOI : 10.1038/s41467-019-11013-2

Résumé

The mechanisms driving the development of extracapillary lesions in focal segmental glomerulosclerosis (FSGS) and crescentic glomerulonephritis (CGN) remain poorly understood. A key question is how parietal epithelial cells (PECs) invade glomerular capillaries, thereby promoting injury and kidney failure. Here we show that expression of the tetraspanin CD9 increases markedly in PECs in mouse models of CGN and FSGS, and in kidneys from individuals diagnosed with these diseases. Cd9 gene targeting in PECs prevents glomerular damage in CGN and FSGS mouse models. Mechanistically, CD9 deficiency prevents the oriented migration of PECs into the glomerular tuft and their acquisition of

CD44 and β1 integrin expression. These findings highlight a critical role for de novo expression of CD9 as a common pathogenic switch driving the PEC phenotype in CGN and FSGS, while offering a potential therapeutic avenue to treat these conditions.

André Görgens, Michel Bremer, Rita Ferrer-Tur, Florian Murke, Tobias Tertel, Peter A Horn, Sebastian Thalmann, Joshua A Welsh, Christine Probst, Coralié Guerin, Chantal M Boulanger, Jennifer C Jones, Helmut Hanenberg, Uta Erdbrügger, Joanne Lannigan, Franz L Ricklefs, Samir El-Andaloussi, Bernd Giebel (2019 Apr 6)
Optimisation of imaging flow cytometry for the analysis of single extracellular vesicles by using fluorescence-tagged vesicles as biological reference material.
Journal of extracellular vesicles : 1587567 : DOI : 10.1080/20013078.2019.1587567

Résumé

Extracellular vesicles (EVs) mediate targeted cellular interactions in normal and pathophysiological conditions and are increasingly recognised as potential biomarkers, therapeutic agents and drug delivery vehicles. Based on their size and biogenesis, EVs are classified as exosomes, microvesicles and apoptotic bodies. Due to overlapping size ranges and the lack of specific markers, these classes cannot yet be distinguished experimentally. Currently, it is a major challenge in the field to define robust and sensitive technological platforms being suitable to resolve EV heterogeneity, especially for small EVs (sEVs) with diameters below 200 nm, i.e. smaller microvesicles and exosomes. Most conventional flow cytometers are not suitable for the detection of particles being smaller than 300 nm, and the poor availability of defined reference materials hampers the validation of sEV analysis protocols. Following initial reports that imaging flow cytometry (IFCM) can be used for the characterisation of larger EVs, we aimed to investigate its usability for the characterisation of sEVs. This study set out to identify optimal sample preparation and instrument settings that would demonstrate the utility of this technology for the detection of single sEVs. By using CD63eGFP-labelled sEVs as a biological reference material, we were able to define and optimise IFCM acquisition and analysis parameters on an Amnis ImageStreamX MkII instrument for the detection of single sEVs. In addition, using antibody-labelling approaches, we show that IFCM facilitates robust detection of different EV and sEV subpopulations in isolated EVs, as well as unprocessed EV-containing samples. Our results indicate that fluorescently labelled sEVs as biological reference material are highly useful for the optimisation of fluorescence-based methods for sEV analysis. Finally, we propose that IFCM will help to significantly increase our ability to assess EV heterogeneity in a rigorous and reproducible manner, and facilitate the identification of specific subsets of sEVs as useful biomarkers in various diseases.

Elisa Rossi, Sonia Poirault-Chassac, Ivan Bieche, Richard Chocron, Anne Schnitzler, Anna Lokajczyk, Pierre Bourdoncle, Blandine Dizier, Nour C Bacha, Nicolas Gendron, Adeline Blandinieres, Coralie L Guerin, Pascale Gaussem, David M Smadja (2019 Mar 18)
Human Endothelial Colony Forming Cells Express Intracellular CD133 that

Modulates their Vasculogenic Properties.
Stem cell reviews and reports : 590-600 : DOI : 10.1007/s12015-019-09881-8

Résumé

Stem cells at the origin of endothelial progenitor cells and in particular endothelial colony forming cells (ECFCs) subtype have been largely supposed to be positive for the CD133 antigen, even though no clear correlation has been established between its expression and function in ECFCs. We postulated that CD133 in ECFCs might be expressed intracellularly, and could participate to vasculogenic properties. ECFCs extracted from cord blood were used either fresh (n = 4) or frozen (n = 4), at culture days <30, to investigate the intracellular presence of CD133 by flow cytometry and confocal analysis. Comparison with HUVEC and HAEC mature endothelial cells was carried out. Then, CD133 was silenced in ECFCs using specific siRNA (siCD133-ECFCs) or scramble siRNA (siCtrl-ECFCs). siCD133-ECFCs (n = 12), siCtrl-ECFCs (n = 12) or PBS (n = 12) were injected in a hind-limb ischemia nude mouse model and vascularization was quantified at day 14 with H&E staining and immunohistochemistry for CD31. Results of flow cytometry and confocal microscopy evidenced the positivity of CD133 in ECFCs after permeabilization compared with not permeabilized ECFCs ($p < 0.001$) and mature endothelial cells ($p < 0.03$). In the model of mouse hind-limb ischemia, silencing of CD133 in ECFCs significantly abolished post-ischemic revascularization induced by siCtrl-ECFCs; indeed, a significant reduction in cutaneous blood flows ($p = 0.03$), capillary density (CD31) ($p = 0.01$) and myofiber regeneration ($p = 0.04$) was observed. Also, a significant necrosis ($p = 0.02$) was observed in mice receiving siCD133-ECFCs compared to those treated with siCtrl-ECFCs. In conclusion, our work describes for the first time the intracellular expression of the stemness marker CD133 in ECFCs. This feature could resume the discrepancies found in the literature concerning CD133 positivity and ontogeny in endothelial progenitors.

Alicja Czopek, Rebecca Moorhouse, Léa Guyonnet, Tariq Farrah, Olivia Lenoir, Elizabeth Owen, Job van Bragt, Hannah M Costello, Filippo Menolascina, Véronique Baudrie, David J Webb, David C Kluth, Matthew A Bailey, Pierre-Louis Tharaux, Neeraj Dhaun (2019 Jan 19)
A novel role for myeloid endothelin-B receptors in hypertension.
European heart journal : 768-784 : DOI : 10.1093/eurheartj/ehy881

Résumé

Hypertension is common. Recent data suggest that macrophages (Mφ) contribute to, and protect from, hypertension. Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor with additional pro-inflammatory properties. We investigated the role of the ET system in experimental and clinical hypertension by modifying Mφ number and phenotype.

Année de publication : 2018

Anna Chipont, Bruno Esposito, Inès Challier, Mélanie Montabord, Alain Tedgui, Ziad Mallat, Xavier Loyer, Stephane Potteaux (2018 Dec 28)

MicroRNA-21 Deficiency Alters the Survival of Ly-6C Monocytes in ApoE Mice and Reduces Early-Stage Atherosclerosis-Brief Report.

Arteriosclerosis, thrombosis, and vascular biology : 170-177 : DOI : 10.1161/ATVBAHA.118.311942

Résumé

Objective- To determine the role of microRNA-21 (miR-21) on the homeostasis of monocyte subsets and on atherosclerosis development in ApoE (apolipoprotein E) mice. Approach and Results- In ApoE mice, miR-21 expression was increased in circulating Ly-6C nonclassical monocytes in comparison to Ly-6C monocytes. The absence of miR-21 significantly altered the survival and number of circulating Ly-6C nonclassical monocytes in ApoE mice. In the early stages of atherosclerosis, the absence of miR-21 limited lesion development both in the aortic sinus (by almost 30%) and in the aorta (by almost 50%). This was associated with less monocyte availability in circulation and increased apoptosis of local macrophages in plaques. At later stages of atherosclerosis, lesion size in the aortic root was similar in ApoE and ApoE miR-21 mice, but plaques showed a less stable phenotype (larger necrotic cores) in the latter. The loss of protection in advanced stages was most likely because of excessive inflammatory apoptosis related to an impairment of local efficient efferocytosis. Conclusions- Gene deletion of miR-21 in ApoE mice alters Ly-6C nonclassical monocytes homeostasis and contribute to limit early-stage atherosclerosis.

Nour C Bacha, Marilyne Levy, Coralie L Guerin, Bernard Le Bonniec, Annie Harroche, Isabelle Szezepanski, Jean M Renard, Pascale Gaussem, Dominique Israel-Biet, Chantal M Boulanger, David M Smadja (2018 Nov 29)

Treprostinil treatment decreases circulating platelet microvesicles and their procoagulant activity in pediatric pulmonary hypertension.

Pediatric pulmonology : 66-72 : DOI : 10.1002/ppul.24190

Résumé

Pulmonary arterial hypertension (PAH) results from pulmonary vascular disease and may eventually lead to right heart failure and death. Vasodilator therapy has greatly improved PAH prognosis. Circulating microvesicles are considered as surrogate markers of endothelial and hematopoietic cell activation.

Marina Wierz, Sandrine Pierson, Ernesto Gargiulo, Coralie Guerin, Etienne Moussay, Jerome Paggetti (2018 Nov 23)

Purification of Leukemia-Derived Exosomes to Study Microenvironment Modulation.
Methods in molecular biology (Clifton, N.J.) : 231-245 : DOI : 10.1007/978-1-4939-8885-3_16

Résumé

Exosomes are membrane-enclosed vesicles released by different cell types into the extracellular space. As mediators of intercellular communication, they are involved in multiple physiological processes, but they are also associated with the pathogenesis of human malignancies including leukemia. Isolation of exosomes enables the characterization of their role in microenvironment modulation as well as their participation in disease pathology. A variety of strategies and techniques exists to purify exosomes from many biological fluids (e.g., blood, urine, and saliva). Here, we describe the efficient production of large quantities of exosomes from leukemic cell lines by using CELLine bioreactors based on two-compartment technology, as well as their isolation and purification by combining differential centrifugation and ultracentrifugation through a density gradient (17% OptiPrep cushion). Thus, exosomes are appropriately prepared for characterization by western blotting to detect exosome markers or imaging flow cytometry (ImageStream), and for downstream analyses such as the internalization in microenvironmental cells by confocal imaging or flow cytometry, methods which are also described in this chapter.

Antoun Al Absi, Hannah Wurzer, Coralie Guerin, Celine Hoffmann, Flora Moreau, Xianqing Mao, Joshua Brown-Clay, Rémi Petrolli, Carla Pou Casellas, Monika Dieterle, Jean-Paul Thiery, Salem Chouaib, Guy Berchem, Bassam Janji, Clément Thomas (2018 Aug 15)
Actin Cytoskeleton Remodeling Drives Breast Cancer Cell Escape from Natural Killer-Mediated Cytotoxicity.
Cancer research : 5631-5643 : DOI : 10.1158/0008-5472.CAN-18-0441

Résumé

Elucidation of the underlying molecular mechanisms of immune evasion in cancer is critical for the development of immunotherapies aimed to restore and stimulate effective antitumor immunity. Here, we evaluate the role of the actin cytoskeleton in breast cancer cell resistance to cytotoxic natural killer (NK) cells. A significant fraction of breast cancer cells responded to NK-cell attack via a surprisingly rapid and massive accumulation of F-actin near the immunologic synapse, a process we termed « actin response. » Live-cell imaging provided direct evidence that the actin response is associated with tumor cell resistance to NK-cell-mediated cell death. High-throughput imaging flow cytometry analyses showed that breast cancer cell lines highly resistant to NK cells were significantly enriched in actin response-competent cells as compared with susceptible cell lines. The actin response was not associated with a defect in NK-cell activation but correlated with reduced intracellular levels of the cytotoxic protease granzyme B and a lower rate of apoptosis in target cells. Inhibition of the actin response by knocking down CDC42 or N-WASP led to a significant increase in granzyme B levels in target cells and was sufficient to convert resistant breast cancer cell lines into a highly susceptible phenotype. The actin response and its protective

effects were fully recapitulated using donor-derived primary NK cells as effector cells. Together, these findings establish the pivotal role of actin remodeling in breast cancer cell resistance to NK-cell-mediated killing. These findings establish the pivotal role of the actin cytoskeleton in driving breast cancer cell resistance to natural killer cells, a subset of cytotoxic lymphocytes with important roles in innate antitumor immunity. .

Xavier Loyer, Ivana Zlatanova, Cecile Devue, Min Yin, Kiave-Yune Howangyin, Phatchanat Klaihmon, Coralie L Guerin, Marouane Kheloufi, Jose Vilar, Konstantinos Zannis, Bernd K Fleischmann, Do Won Hwang, Jongmin Park, Hakho Lee, Philippe Menasché, Jean-Sébastien Silvestre, Chantal M Boulanger (2018 Mar 30)

Intra-Cardiac Release of Extracellular Vesicles Shapes Inflammation Following Myocardial Infarction.

Circulation research : 100-106 : DOI : 10.1161/CIRCRESAHA.117.311326

Résumé

A rapid and massive influx of inflammatory cells occurs into ischemic area after myocardial infarction (MI), resulting in local release of cytokines and growth factors. Yet, the mechanisms regulating their production are not fully explored. The release of extracellular vesicles (EVs) in the interstitial space curbs important biological functions, including inflammation, and influences the development of cardiovascular diseases. To date, there is no evidence for in situ release of cardiac EVs after MI.