A compendium of single extracellular vesicle flow cytometry

Type	Article de revue
Auteur	Joshua A. Welsh
Auteur	Ger J. A. Arkesteijn
Auteur	Michel Bremer
Auteur	Michael Cimorelli
Auteur	Françoise Dignat-George
Auteur	Bernd Giebel
Auteur	André Görgens
Auteur	An Hendrix
Auteur	Martine Kuiper
Auteur	Romaric Lacroix
Auteur	Joanne Lannigan
Auteur	Ton G. van Leeuwen
Auteur	Estefanía Lozano-Andrés
Auteur	Shoaib Rao
Auteur	Stéphane Robert
Auteur	Leonie de Rond
Auteur	Vera A. Tang
Auteur	Tobias Tertel
Auteur	Xiaomei Yan
Auteur	Marca H. M. Wauben
Auteur	John P. Nolan
Auteur	Jennifer C. Jones
Auteur	Rienk Nieuwland
Auteur	Edwin van der Pol
Résumé	Flow cytometry (FCM) offers a multiparametric technology capable of characterizing single extracellular vesicles (EVs). However, most flow cytometers are designed to detect cells, which are larger than EVs. Whereas cells exceed the background noise, signals originating from EVs partly overlap with the background noise, thereby making EVs more difficult to detect than cells. This technical mismatch together with complexity of EV-containing fluids causes limitations and challenges with conducting, interpreting and reproducing EV FCM experiments. To address and overcome these challenges, researchers from the International Society for Extracellular Vesicles (ISEV), International Society for Advancement of Cytometry (ISAC), and the International Society on Thrombosis and Haemostasis (ISTH) joined forces and initiated the EV FCM working group. To improve the interpretation, reporting, and reproducibility of future EV FCM data, the EV FCM working group published an ISEV position manuscript outlining a framework of minimum information that should be reported about an

	FCM experiment on single EVs (MIFlowCyt-EV). However, the framework contains limited background information. Therefore, the goal of this compendium is to provide the background information necessary to design and conduct reproducible EV FCM experiments. This compendium contains background information on EVs, the interaction between light and EVs, FCM hardware, experimental design and preanalytical procedures, sample preparation, assay controls, instrument data acquisition and calibration, EV characterization, and data reporting. Although this compendium focuses on EVs, many concepts and explanations could also be applied to FCM detection of other particles within the EV size range, such as bacteria, lipoprotein particles, milk fat globules, and viruses.
Date	2023
Langue	en
Catalogue de bibl.	Wiley Online Library
URL	http://onlinelibrary.wiley.com/doi/abs/10.1002/jev2.12299
Consulté le	20/02/2023 à 11:42:21
Extra	_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jev2.12299
Volume	12
Pages	e12299
Publication	Journal of Extracellular Vesicles
DOI	10.1002/jev2.12299
Numéro	2
ISSN	2001-3078
Date d'ajout	20/02/2023 à 11:42:21
Modifié le	20/02/2023 à 11:42:21

- **Marqueurs :**
  - extracellular vesicles
  - microparticles
  - standardization
  - nanoparticles
  - flow cytometry
  - calibration
  - MIFlowCyt-EV

Pièces jointes

  - Full Text PDF
  - Snapshot

- # A new assay to evaluate microvesicle plasmin generation capacity: validation in disease with fibrinolysis imbalance

Type	Article de revue
Auteur	Sylvie Cointe
Auteur	Karim Harti Souab
Auteur	Tarik Bouriche
Auteur	Loris Vallier
Auteur	Amandine Bonifay
Auteur	Coralie Judicone
Auteur	Stéphane Robert
Auteur	Romain Armand
Auteur	Philippe Poncelet
Auteur	Jacques Albanese
Auteur	Françoise Dignat-George
Auteur	Romaric Lacroix
Résumé	Among extracellular vesicles, leukocyte-derived microvesicles (LMVs) have emerged as complex vesicular structures. Primarily identified as procoagulant entities, they were more recently ascribed to plasmin generation capacity (MV-PGC). The objectives of this work were (1) to develop a new hybrid bio-assay combining the specific isolation of LMVs and measurement of their PGC, and compare its performance to the original method based on centrifugation, (2) to validate MV-PGC in septic shock, combining increased levels of LMVs and fibrinolytic imbalance. Using plasma sample spiked with LMVs featuring different levels of PGC, we demonstrated that CD15-beads specifically extracted LMVs. The MV dependency of the test was demonstrated using electron microscopy, high speed centrifugation, nanofiltration and detergent-mediated solubilization and the MV-PGC specificity using plasmin-specific inhibitors, or antibodies blocking elastase or uPA. Thanks to a reaction booster (ε-ACA), we showed that the assay was more sensitive and reproducible than the original method. Moreover, it exhibited a good repeatability, inter-operator and inter-experiment reproducibility. The new immunomagnetic bio-assay was further validated in patients with septic shock. As a result, we showed that MV-PGC values were significantly lower in septic shock patients who died compared to patients who survived, both at inclusion and 24 h later (1.4 [0.8-3.0] vs 3.1 [1.7-18] A405 × 10-3/min, p = 0.02; 1.4 [1-1.6] vs 5.2 [2.2-16] A405 × 10-3/min, p = 0.004). Interestingly, combining both MV-PGC and PAI-1 in a ratio significantly improved the predictive value of PAI-1. This strategy, a hybrid capture bioassay to specifically measure LMV-PGC using for the first time, opens new perspectives for measuring subcellular fibrinolytic potential in clinical settings with fibrinolytic imbalance.
Date	2018
Langue	eng
Titre abrégé	A new assay to evaluate microvesicle plasmin generation capacity

Catalogue de bibl.	PubMed
Extra	Number: 1
Volume	7
Pages	1494482
Publication	Journal of Extracellular Vesicles
DOI	10.1080/20013078.2018.1494482
Numéro	1
Abrév. de revue	J Extracell Vesicles
ISSN	2001-3078
Date d'ajout	13/12/2021 à 10:35:05
Modifié le	13/12/2021 à 10:35:05

- **Marqueurs :**
  - extracellular vesicles
  - Fibrinolysis
  - Microvesicles
  - immunomagnetic separation
  - septic shock

Pièces jointes

  - PubMed entry
  - Texte intégral

- # A new strategy to count and sort neutrophil-derived extracellular vesicles: Validation in infectious disorders

Type	Article de revue
Auteur	Amandine Bonifay
Auteur	Stéphane Robert
Auteur	Belinda Champagne
Auteur	Paul-Rémi Petit
Auteur	Aude Eugène
Auteur	Corinne Chareyre
Auteur	Anne-Claire Duchez
Auteur	Mélanie Vélier
Auteur	Shirley Fritz
Auteur	Loris Vallier
Auteur	Romaric Lacroix
Auteur	Françoise Dignat-George

Résumé	Newly recognized polymorphonuclear neutrophil (PMNs) functions include the ability to release subcellular mediators such as neutrophil-derived extracellular vesicles (NDEVs) involved in immune and thrombo-inflammatory responses. Elevation of their plasmatic level has been reported in a variety of infectious and cardiovascular disorders, but the clinical use of this potential biomarker is hampered by methodological issues. Although flow cytometry (FCM) is currently used to detect NDEVs in the plasma of patients, an extensive characterization of NDEVs has never been done. Moreover, their detection remains challenging because of their small size and low antigen density. Therefore, the objective of the present study was first to establish a surface antigenic signature of NDEVs detectable by FCM and therefore to improve their detection in biological fluids by developing a strategy allowing to overcome their low fluorescent signal and reduce the background noise. By testing a large panel of 54 antibody specificities already reported to be positive on PMNs, we identified a profile of 15 membrane protein markers, including 4 (CD157, CD24, CD65 and CD66c) never described on NDEVs. Among them, CD15, CD66b and CD66c were identified as the most sensitive and specific markers to detect NDEVs by FCM. Using this antigenic signature, we developed a new strategy combining the three best antibodies in a cocktail and reducing the background noise by size exclusion chromatography (SEC). This strategy allowed a significant improvement in NDEVs enumeration in plasma from sepsis patients and made it feasible to efficiently sort NDEVs from COVID-19 patients. Altogether, this work opens the door to a more valuable measurement of NDEVs as a potential biomarker in clinical practice. A similar strategy could also be applied to improve detection by FCM of other rare subpopulations of EVs generated by tissues with limited access, such as vascular endothelium, cancer cells or placenta.
Date	2022
Langue	en
Titre abrégé	A new strategy to count and sort neutrophil-derived extracellular vesicles
Catalogue de bibl.	Wiley Online Library
URL	https://onlinelibrary.wiley.com/doi/abs/10.1002/jev2.12204
Consulté le	01/04/2022 à 15:43:11
Extra	_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jev2.12204
Volume	11
Pages	e12204
Publication	Journal of Extracellular Vesicles
DOI	10.1002/jev2.12204
Numéro	4
ISSN	2001-3078
Date d'ajout	01/04/2022 à 15:43:11

Modifié le 01/04/2022 à 15:43:11

- **Marqueurs :**
  - extracellular vesicles
  - flow cytometry
  - EVs sorting
  - infectious associated diseases
  - neutrophils
  - size exclusion chromatography

Pièces jointes

  - Full Text PDF
  - Snapshot

- # A novel anti-CD146 antibody specifically targets cancer cells by internalizing the molecule

Type	Article de revue
Auteur	Marie Nollet
Auteur	Jimmy Stalin
Auteur	Anaïs Moyon
Auteur	Waël Traboulsi
Auteur	Amel Essaadi
Auteur	Stéphane Robert
Auteur	Nausicaa Malissen
Auteur	Richard Bachelier
Auteur	Laurent Daniel
Auteur	Alexandrine Foucault-Bertaud
Auteur	Caroline Gaudy-Marqueste
Auteur	Romaric Lacroix
Auteur	Aurélie S. Leroyer
Auteur	Benjamin Guillet
Auteur	Nathalie Bardin
Auteur	Françoise Dignat-George
Auteur	Marcel Blot-Chabaud
Résumé	CD146 is an adhesion molecule present on many tumors (melanoma, kidney, pancreas, breast, ...). In addition, it has been shown to be expressed on vascular endothelial and smooth muscle cells. Generating an antibody able to specifically recognize CD146 in cancer cells (designated as tumor CD146), but not in normal cells, would thus be of major interest for targeting tumor CD146 without affecting the vascular system. We thus generated antibodies against the extracellular domain of the molecule produced in cancer cells and selected an antibody that specifically recognizes tumor CD146. This antibody (TsCD146 mAb)

was able to detect CD146-positive tumors in human biopsies and in vivo, by PET imaging, in a murine xenograft model. In addition, TsCD146 mAb antibody was able to specifically detect CD146-positive cancer microparticles in the plasma of patients. TsCD146 mAb displayed also therapeutic effects since it was able to reduce the growth of human CD146-positive cancer cells xenografted in nude mice. This effect was due to a decrease in the proliferation and an increase in the apoptosis of CD146-positive cancer cells after TsCD146-mediated internalization of the cell surface CD146. Thus, TsCD146 mAb could be of major interest for diagnostic and therapeutic strategies against CD146-positive tumors in a context of personalized medicine.

Date	Dec 22, 2017
Langue	eng
Catalogue de bibl.	PubMed
Extra	Number: 68
Volume	8
Pages	112283-112296
Publication	Oncotarget
DOI	10.18632/oncotarget.22736
Numéro	68
Abrév. de revue	Oncotarget
ISSN	1949-2553
Date d'ajout	13/12/2021 à 10:35:05
Modifié le	13/12/2021 à 10:35:05

- **Marqueurs :**
  - antibody
  - cancer
  - CD146
  - PET
  - therapy

Pièces jointes

  - PubMed entry
  - Texte intégral

- # Biogenesis of Pro-senescent Microparticles by Endothelial Colony Forming Cells from Premature Neonates is driven by SIRT1-Dependent Epigenetic Regulation of MKK6

Type	Article de revue

Auteur	Stéphanie Simoncini
Auteur	Anne-Line Chateau
Auteur	Stéphane Robert
Auteur	Dilyana Todorova
Auteur	Catherine Yzydorzick
Auteur	Romaric Lacroix
Auteur	Isabelle Ligi
Auteur	Laurence Louis
Auteur	Richard Bachelier
Auteur	Umberto Simeoni
Auteur	Frédérique Magdinier
Auteur	Françoise Dignat-George
Auteur	Florence Sabatier

Résumé	Senescent cells may exert detrimental effect on microenvironment through the secretion of soluble factors and the release of extracellular vesicles, such as microparticles, key actors in ageing and cardiovascular diseases. We previously reported that sirtuin-1 (SIRT1) deficiency drives accelerated senescence and dysfunction of endothelial colony-forming cells (ECFC) in PT neonates. Because preterm birth (PT) increases the risk for cardiovascular diseases during neonatal period as well as at adulthood, we hypothesized that SIRT1 deficiency could control the biogenesis of microparticles as part of a senescence-associated secretory phenotype (SASP) of PT-ECFC and investigated the related molecular mechanisms. Compared to control ECFC, PT-ECFC displayed a SASP associated with increased release of endothelial microparticles (EMP), mediating a paracrine induction of senescence in naïve endothelial cells. SIRT1 level inversely correlated with EMP release and drives PT-ECFC vesiculation. Global transcriptomic analysis revealed changes in stress response pathways, specifically the MAPK pathway. We delineate a new epigenetic mechanism by which SIRT1 deficiency regulates MKK6/p38MAPK/Hsp27 pathway to promote EMP biogenesis in senescent ECFC. These findings deepen our understanding of the role of ECFC senescence in the disruption of endothelial homeostasis and provide potential new targets towards the control of cardiovascular risk in individuals born preterm.

Date	08 15, 2017
Langue	eng
Catalogue de bibl.	PubMed
Extra	Number: 1
Volume	7
Pages	8277
Publication	Scientific Reports
DOI	10.1038/s41598-017-08883-1

Numéro	1
Abrév. de revue	Sci Rep
ISSN	2045-2322
Date d'ajout	13/12/2021 à 10:35:05
Modifié le	13/12/2021 à 10:35:05

- **Marqueurs :**
  - Humans
  - Models, Biological
  - Cell-Derived Microparticles
  - Endothelial Cells
  - Signal Transduction
  - Cellular Senescence
  - Gene Expression Profiling
  - Transcriptome
  - Gene Expression Regulation
  - Endothelial Progenitor Cells
  - Epigenesis, Genetic
  - Gene Deletion
  - Infant, Newborn
  - MAP Kinase Kinase 6
  - p38 Mitogen-Activated Protein Kinases
  - Paracrine Communication
  - Premature Birth
  - Sirtuin 1

Pièces jointes

  - PubMed entry
  - Texte intégral

- # CD146 deficiency promotes plaque formation in a mouse model of atherosclerosis by enhancing RANTES secretion and leukocyte recruitment

Type	Article de revue
Auteur	Muriel G. Blin
Auteur	Richard Bachelier
Auteur	Karim Fallague
Auteur	Karima Moussouni
Auteur	Michel Aurrand-Lions
Auteur	Samantha Fernandez
Auteur	Benjamin Guillet
Auteur	Stéphane Robert
Auteur	Alexandrine Foucault-Bertaud

Auteur Nathalie Bardin
Auteur Marcel Blot-Chabaud
Auteur Françoise Dignat-George
Auteur Aurélie S. Leroyer

Résumé AIMS: The progression of atherosclerosis is based on the continued recruitment of leukocytes in the vessel wall. The previously described role of CD146 in leukocyte infiltration suggests an involvement for this adhesion molecule in the inflammatory response. In this study, we investigated the role of CD146 in leukocyte recruitment by using an experimental model of atherogenesis. METHODS AND RESULTS: The role of CD146 was explored in atherosclerosis by crossing CD146-/- mice with ApoE-/- mice. CD146 -/-/ApoE -/- and ApoE -/- mice were fed a Western diet for 24 weeks and were monitored for aortic wall thickness using high frequency ultrasound. The arterial wall was significantly thicker in CD146-deficient mice. After 24 weeks of Western diet, a significant increase of atheroma in both total aortic lesion and aortic sinus of CD146-null mice was observed. In addition, atherosclerotic lesions were more inflammatory since plaques from CD146-deficient mice contained more neutrophils and macrophages. This was due to up-regulation of RANTES secretion by macrophages in CD146-deficient atherosclerotic arteries. This prompted us to further address the function of CD146 in leukocyte recruitment during acute inflammation by using a second experimental model of peritonitis induced by thioglycollate. Neutrophil recruitment was significantly increased in CD146-deficient mice 12 h after peritonitis induction and associated with higher RANTES levels in the peritoneal cavity. In CD146-null macrophages, we also showed that increased RANTES production was dependent on constitutive inhibition of the p38-MAPK signaling pathway. Finally, Maraviroc, a RANTES receptor antagonist, was able to reduce atherosclerotic lesions and neutrophilia in CD146-deficient mice to the same level as that found in ApoE -/- mice. CONCLUSIONS: Our data indicate that CD146 deficiency is associated with the upregulation of RANTES production and increased inflammation of atheroma, which could influence the atherosclerotic plaque fate. Thus, these data identify CD146 agonists as potential new therapeutic candidates for atherosclerosis treatment.

Date 05 2019
Langue eng
Catalogue de bibl. PubMed
Volume 130
Pages 76-87
Publication Journal of Molecular and Cellular Cardiology
DOI 10.1016/j.yjmcc.2019.03.017
Abrév. de revue J. Mol. Cell. Cardiol.
ISSN 1095-8584

Date d'ajout	13/12/2021 à 10:35:04
Modifié le	13/12/2021 à 10:35:04

- **Pièces jointes**
  - PubMed entry
  - Version soumise

Extracellular vesicles from T cells overexpress miR-146b-5p in HIV-1 infection and repress endothelial activation

Type	Article de revue
Auteur	Estelle Balducci
Auteur	Aurélie S. Leroyer
Auteur	Romaric Lacroix
Auteur	Stéphane Robert
Auteur	Dilyana Todorova
Auteur	Stéphanie Simoncini
Auteur	Luc Lyonnet
Auteur	Corinne Chareyre
Auteur	Olivia Zaegel-Faucher
Auteur	Joëlle Micallef
Auteur	Isabelle Poizot-Martin
Auteur	Patrice Roll
Auteur	Françoise Dignat-George
Résumé	Human immunodeficiency virus type 1 (HIV-1) infection promotes a generalized activation of host responses that involves not only CD4 T cells, but also cells of the microenvironment, which are not directly infected, such as endothelial cells. The mechanisms triggering HIV-1-associated vascular alterations remain poorly understood. Extracellular vesicles (EVs), implicated in cell-to-cell communication, have been recently described as carriers of microRNAs (miRNAs). Here, we show that miR-146b-5p is upregulated in both CD4 T cells, CD4 T cell-derived EVs and circulating EVs obtained from antiretroviral therapy-naive HIV-1-infected patients. We further demonstrate that EVs from T cell line overexpressing miR-146b-5p mimics (miR-146b-EVs): 1) protect their miRNA cargo from RNase degradation, 2) transfer miR-146b-5p mimics into endothelial cells and 3) reduce endothelial inflammatory responses in vitro and in vivo in the lungs of mice through the downregulation of nuclear factor-κB-responsive molecules. These data advance our understanding on chronic inflammatory responses affecting endothelial homeostasis, in infectious and non-infectious diseases and pave the way for potential new anti-inflammatory strategies.
Date	07 16, 2019

Langue	eng
Catalogue de bibl.	PubMed
Extra	Number: 1
Volume	9
Pages	10299
Publication	Scientific Reports
DOI	10.1038/s41598-019-44743-w
Numéro	1
Abrév. de revue	Sci Rep
ISSN	2045-2322
Date d'ajout	13/12/2021 à 10:34:50
Modifié le	13/12/2021 à 10:34:50

- **Pièces jointes**
  - PubMed entry
  - Texte intégral

Granulocyte microvesicles with a high plasmin generation capacity promote clot lysis and improve outcome in septic shock

Type	Article de revue
Auteur	Sylvie Cointe
Auteur	Loris Vallier
Auteur	Pierre Esnault
Auteur	Mathilde Dacos
Auteur	Amandine Bonifay
Auteur	Nicolas Macagno
Auteur	Karim Harti Souab
Auteur	Corinne Chareyre
Auteur	Coralie Judicone
Auteur	Diane Frankel
Auteur	Stephane Robert
Auteur	Sami Hraiech
Auteur	Marie-Christine Alessi
Auteur	Philippe Poncelet
Auteur	Jacques Albanese
Auteur	Françoise Dignat-George
Auteur	Romaric Lacroix
Résumé	Microvesicles (MVs) have previously been shown to exert profibrinolytic capacity, which is increased in patients with septic

shock (SS) with a favorable outcome. We therefore hypothesized that the plasmin generation capacity (PGC) could confer to MVs a protective effect supported by their capacity to lyse a thrombus, and we investigated the mechanisms involved. Using a MV-PGC kinetic assay, ELISA and flow cytometry, we found that granulocyte MVs (Gran-MVs) from SS patients display a heterogeneous PGC profile driven by the uPA (urokinase)/uPAR system. In vitro, these MVs lyse a thrombus according to their MV-PGC levels in a uPA/uPAR-dependent manner, as shown in a fluorescent clot lysis test and a lysis front retraction assay. Fibrinolytic activators conveyed by MVs contribute to approximately 30% of the plasma plasminogenolytic capacity of SS patients. In a murine model of SS, the injection of high PGC Gran-MVs significantly improved mouse survival and reduced the number of thrombi in vital organs. This was associated with a modification of the mouse coagulation and fibrinolysis properties toward a more fibrinolytic profile. Interestingly, mouse survival was not improved when soluble uPA was injected. Finally, using a multiplex array on plasma from SS patients, we found that neutrophil elastase correlates with the effect of high-PGC-capacity plasma and modulates the Gran-MV plasmin generation capacity by cleaving uPA-PAI-1 complexes. In conclusion, we show that high PGC level displayed by Gran-MVs reduce thrombus formation and improve survival conferring to Gran-MVs a protective role in a murine model of sepsis.

Date	2022-01-13
Langue	eng
Catalogue de bibl.	PubMed
Extra	PMID: 35026004
Pages	blood.2021013328
Publication	Blood
DOI	10.1182/blood.2021013328
Abrév. de revue	Blood
ISSN	1528-0020
Date d'ajout	17/01/2022 à 17:30:44
Modifié le	17/01/2022 à 17:30:44

- **Pièces jointes**
  - PubMed entry

Immune thrombotic thrombocytopenic purpura plasmas induce calcium- and IgG-dependent endothelial activation: correlations with disease severity

Type	Article de revue

Auteur Edwige Tellier
Auteur Agnès Widemann
Auteur Raphaël Cauchois
Auteur Julien Faccini
Auteur Marie Lagarde
Auteur Marion Brun
Auteur Philippe Robert
Auteur Stéphane Robert
Auteur Richard Bachelier
Auteur Pascale Poullin
Auteur Elien Roose
Auteur Karen Vanhoorelbeke
Auteur Paul Coppo
Auteur Françoise Dignat-George
Auteur Gilles Kaplanski

Résumé Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is characterized by a severe ADAMTS13 deficiency due to the presence of anti-ADAMTS13 autoantibodies, with subsequent accumulation of circulating ultra-large von Willebrand Factor (VWF) multimers. The role of endothelial cell activation as a trigger of the disease has been suggested in animal models but remains to be demonstrated in humans. We prospectively obtained plasma from the first plasma exchange of 25 patients during iTTP acute phase. iTTP but not control plasma, induced a rapid VWF release and P-selectin exposure on dermal human micro-vascular endothelial cell (HMVEC-d) surface, associated with angiopoietin-2 and endothelin-1 secretion, consistent with Weibel-Palade bodies exocytosis. Calcium (Ca2+) blockade significantly decreased VWF release, whereas iTTP plasma induced a rapid and sustained Ca2+ flux in HMVEC-d which correlated in retrospect, with disease severity and survival in 62 iTTP patients. F(ab)'2 fragments purified from the immunoglobulin G (IgG) fraction of iTTP plasma mainly induced endothelial cell (EC) activation with additional minor roles for circulating free heme and nucleosomes, but not for complement. Furthermore, two anti-ADAMTS13 monoclonal antibodies purified from iTTP patient B cells, but not serum from hereditary TTP, induced endothelial Ca2+ flux associated with Weibel-Palade bodies exocytosis in vitro, whereas inhibition of endothelial ADAMTS13 expression using small interference RNA, significantly decreased the stimulating effects of iTTP IgG. In conclusion, Ca2+-mediated endothelial cell activation constitutes a second "hit" of iTTP, is correlated with the severity of the disease and may constitute a possible therapeutic target.

Date 2022-12-01
Langue eng
Titre abrégé Immune thrombotic thrombocytopenic purpura plasmas induce calcium- and IgG-dependent endothelial activation

Catalogue de bibl.	PubMed
Extra	PMID: 36453103
Publication	Haematologica
DOI	10.3324/haematol.2022.280651
Abrév. de revue	Haematologica
ISSN	1592-8721
Date d'ajout	12/01/2023 à 09:19:06
Modifié le	12/01/2023 à 09:19:06

- **Pièces jointes**
  - PubMed entry
  - Texte intégral

- # MIFlowCyt-EV: a framework for standardized reporting of extracellular vesicle flow cytometry experiments

Type	Article de revue
Auteur	Joshua A. Welsh
Auteur	Edwin Van Der Pol
Auteur	Ger J. A. Arkesteijn
Auteur	Michel Bremer
Auteur	Alain Brisson
Auteur	Frank Coumans
Auteur	Françoise Dignat-George
Auteur	Erika Duggan
Auteur	Ionita Ghiran
Auteur	Bernd Giebel
Auteur	André Görgens
Auteur	An Hendrix
Auteur	Romaric Lacroix
Auteur	Joanne Lannigan
Auteur	Sten F. W. M. Libregts
Auteur	Estefanía Lozano-Andrés
Auteur	Aizea Morales-Kastresana
Auteur	Stephane Robert
Auteur	Leonie De Rond
Auteur	Tobias Tertel
Auteur	John Tigges
Auteur	Olivier De Wever

Auteur	Xiaomei Yan
Auteur	Rienk Nieuwland
Auteur	Marca H. M. Wauben
Auteur	John P. Nolan
Auteur	Jennifer C. Jones

Résumé	Extracellular vesicles (EVs) are small, heterogeneous and difficult to measure. Flow cytometry (FC) is a key technology for the measurement of individual particles, but its application to the analysis of EVs and other submicron particles has presented many challenges and has produced a number of controversial results, in part due to limitations of instrument detection, lack of robust methods and ambiguities in how data should be interpreted. These complications are exacerbated by the field's lack of a robust reporting framework, and many EV-FC manuscripts include incomplete descriptions of methods and results, contain artefacts stemming from an insufficient instrument sensitivity and inappropriate experimental design and lack appropriate calibration and standardization. To address these issues, a working group (WG) of EV-FC researchers from ISEV, ISAC and ISTH, worked together as an EV-FC WG and developed a consensus framework for the minimum information that should be provided regarding EV-FC. This framework incorporates the existing Minimum Information for Studies of EVs (MISEV) guidelines and Minimum Information about a FC experiment (MIFlowCyt) standard in an EV-FC-specific reporting framework (MIFlowCyt-EV) that supports reporting of critical information related to sample staining, EV detection and measurement and experimental design in manuscripts that report EV-FC data. MIFlowCyt-EV provides a structure for sharing EV-FC results, but it does not prescribe specific protocols, as there will continue to be rapid evolution of instruments and methods for the foreseeable future. MIFlowCyt-EV accommodates this evolution, while providing information needed to evaluate and compare different approaches. Because MIFlowCyt-EV will ensure consistency in the manner of reporting of EV-FC studies, over time we expect that adoption of MIFlowCyt-EV as a standard for reporting EV-FC studies will improve the ability to quantitatively compare results from different laboratories and to support the development of new instruments and assays for improved measurement of EVs.

Date	2020
Langue	eng
Titre abrégé	MIFlowCyt-EV
Catalogue de bibl.	PubMed
Extra	Number: 1
Volume	9
Pages	1713526
Publication	Journal of Extracellular Vesicles
DOI	10.1080/20013078.2020.1713526

Numéro	1
Abrév. de revue	J Extracell Vesicles
ISSN	2001-3078
Date d'ajout	13/12/2021 à 10:36:14
Modifié le	13/12/2021 à 10:36:14

- **Marqueurs :**
  - extracellular vesicles
  - Flow Cytometry
  - standardization
  - framework
  - reporting

Pièces jointes

  - PubMed entry
  - Texte intégral

- # Natural Killer Cells Exhibit a Peculiar Phenotypic Profile in Systemic Sclerosis and Are Potent Inducers of Endothelial Microparticles Release

Type	Article de revue
Auteur	Audrey Benyamine
Auteur	Jérémy Magalon
Auteur	Florence Sabatier
Auteur	Luc Lyonnet
Auteur	Stéphane Robert
Auteur	Chloé Dumoulin
Auteur	Sophie Morange
Auteur	Karin Mazodier
Auteur	Gilles Kaplanski
Auteur	Martine Reynaud-Gaubert
Auteur	Pascal Rossi
Auteur	Françoise Dignat-George
Auteur	Brigitte Granel
Auteur	Pascale Paul
Résumé	The pathophysiology of systemic sclerosis (SSc) involves early endothelial and immune activation, both preceding the onset of fibrosis. We previously identified soluble fractalkine and circulating endothelial microparticles (EMPs) as biomarkers of endothelial inflammatory activation in SSc. Fractalkine plays a dual role as a membrane-bound adhesion molecule expressed in inflamed endothelial cells (ECs) and as

a chemokine involved in the recruitment, transmigration, and cytotoxic activation of immune cells that express CX3CR1, the receptor of fractalkine, namely CD8 and γδ T cells and natural killer (NK) cells. We aimed to quantify circulating cytotoxic immune cells and their expression of CX3CR1. We further investigated the expression profile of NK cells chemokine receptors and activation markers and the potential of NK cells to induce EC activation in SSc. We performed a monocentric study (NCT 02636127) enrolling 15 SSc patients [15 females, median age of 55 years (39-63), 11 limited cutaneous form and 4 diffuse] and 15 healthy controls. Serum fractalkine levels were significantly increased in SSc patients. Circulating CD8 T cells numbers were decreased in SSc patients with no difference in their CX3CR1 expression. Circulating γδ T cells and NK cells numbers were preserved. CX3CR1 expression in CD8 and γδ T cells did not differ between SSc patients and controls. The percentage and level of CX3CR1 expression in NK cells were significantly lowered in SSc patients. Percentages of CXCR4, NKG2D, CD69-expressing NK cells, and their expression levels were decreased in NK cells. Conversely, CD16 level expression and percentages of CD16+ NK cells were preserved. The exposure of human microvascular dermic EC line (HMVEC-d) to peripheral blood mononuclear cells resulted in similar NK cells degranulation activity in SSc patients and controls. We further showed that NK cells purified from the blood of SSc patients induced enhanced release of EMPs than NK cells from controls. This study evidenced a peculiar NK cells phenotype in SSc characterized by decreased chemokine and activation receptors expression, that might reflect NK cells involvement in the pathogenic process. It also highlighted the role of NK cells as a potent mechanism inducing endothelial activation through enhanced EMPs release.

Date	2018
Langue	eng
Catalogue de bibl.	PubMed
Volume	9
Pages	1665
Publication	Frontiers in Immunology
DOI	10.3389/fimmu.2018.01665
Abrév. de revue	Front Immunol
ISSN	1664-3224
Date d'ajout	13/12/2021 à 10:35:05
Modifié le	13/12/2021 à 10:35:05

- **Marqueurs :**
  - CX3CR1
  - endothelial microparticles
  - fractalkine

- natural killer cells
- systemic sclerosis

Pièces jointes

- PubMed entry
- Texte intégral

- # Neutrophil extracellular traps are associated with the pathogenesis of diffuse alveolar hemorrhage in murine lupus

Type	Article de revue
Auteur	Pierre-André Jarrot
Auteur	Edwige Tellier
Auteur	Lea Plantureux
Auteur	Lydie Crescence
Auteur	Stéphane Robert
Auteur	Corinne Chareyre
Auteur	Laurent Daniel
Auteur	Véronique Secq
Auteur	Stéphane Garcia
Auteur	Françoise Dignat-George
Auteur	Laurence Panicot-Dubois
Auteur	Christophe Dubois
Auteur	Gilles Kaplanski
Résumé	Diffuse alveolar hemorrhage (DAH) is a life-threatening complication of systemic lupus erythematosus (SLE) and systemic vasculitis. Although initially described to have antibacterial properties, increasing evidence suggests that neutrophil extracellular traps (NETs) have a detrimental role in both autoimmune diseases and acute lung injury. We investigated whether NETs could be detected in a murine model of pristane-induced lupus DAH and contribute to lung injury. Such NETs might constitute a therapeutic target. NETs were characterized by immunofluorescence staining of DNA, neutrophil elastase and citrullinated histones. Evaluation of lung injury was performed by haematoxylin-eosin staining and a quantification program. Clinical status of the mice was assessed by measurement of arterial oxygen saturation and survival curves after recombinant human deoxyribonuclease-1 (Rh-DNase-1) inhalations or polymorphonuclear neutrophil (PMN) depletion. Pristane was found to promote NETs formation in vitro and in vivo. Treatment of mice with Rh-DNase-1 inhalations cleared NETs and reduced lung injury. Clinical status improved significantly, with increased arterial oxygenation and survival. Following PMN depletion, NETs were absent with a subsequent reduction of lung injury and improved arterial oxygenation.

	These results support a pathogenic role of PMNs and NETs in lung injury during pristane-induced DAH. Targeting NETs with Rh-DNase-1 inhalations could constitute an interesting adjuvant therapy in human DAH.
Date	Jun 2019
Langue	eng
Catalogue de bibl.	PubMed
Volume	100
Pages	120-130
Publication	Journal of Autoimmunity
DOI	10.1016/j.jaut.2019.03.009
Abrév. de revue	J. Autoimmun.
ISSN	1095-9157
Date d'ajout	13/12/2021 à 10:34:50
Modifié le	13/12/2021 à 10:34:50

- **Marqueurs :**
  - Neutrophil extracellular traps
  - Deoxyribonuclease-1
  - Diffuse alveolar hemorrhage
  - Murine model
  - Systemic lupus erythematosus

Pièces jointes

  - PubMed entry

- # Oral Squamous Cell Carcinoma Is Associated with a Low Thrombosis Risk Due to Storage Pool Deficiency in Platelets

Type	Article de revue
Auteur	Pierre Haen
Auteur	Lydie Crescence
Auteur	Diane Mege
Auteur	Alexandre Altié
Auteur	Christophe Dubois
Auteur	Laurence Panicot-Dubois
Résumé	Venous thrombo-embolism (VTE) disease is the second most common cause of mortality in cancer patients, and evaluation and prevention of thrombosis risk is essential. VTE-associated risk varies according to the type of tumor disease. Oral cancer is the most frequent type of head and neck cancer, and it represents approximately 2.1% of all cancers

worldwide. Most tumors are squamous cell carcinomas and are mainly due to tobacco and alcohol abuse. VTE risk associated with oral squamous cell carcinoma (OSCC) is low. However, many studies have shown that OSCC has the following biological features of cancers associated with a high thrombosis risk: modified thrombosis and fibrinolysis mechanisms; strong expression of procoagulant proteins; secretion of procoagulant microparticles; and production of procoagulant cytokines. Using an original mouse model of tongue squamous cell carcinoma, our study aimed to clarify this paradoxical situation. First, we showed that OSCC tumors have a pro-aggregatory phenotype and a high local thrombosis risk. Second, we found that tongue tumor mice do not have an elevated systemic thrombosis risk (the risk of an "at distance" thrombosis event such as lower extremity deep venous thrombosis or pulmonary embolism) and even show a reduction in risk. Third, we demonstrated that tongue tumor mice show a reduction in platelet reactivity, which explains the low systemic thrombosis risk. Finally, we found that tongue tumor mice present granule pool deficiency, thereby explaining the reduction in platelet reactivity and systemic thrombosis risk.

Date	2021-02-24
Langue	eng
Catalogue de bibl.	PubMed
Extra	Number: 3 PMID: 33668375 PMCID: PMC7996194
Volume	9
Pages	228
Publication	Biomedicines
DOI	10.3390/biomedicines9030228
Numéro	3
Abrév. de revue	Biomedicines
ISSN	2227-9059
Date d'ajout	13/12/2021 à 10:37:53
Modifié le	13/12/2021 à 10:37:53

- **Marqueurs :**
  - disorders of platelet function
  - intravital microcopy
  - laser injury
  - oral squamous cancer
  - platelet granules

Pièces jointes

- PubMed entry
- Texte intégral

- # Soluble CD146 as a Potential Target for Preventing Triple Negative Breast Cancer MDA-MB-231 Cell Growth and Dissemination

Type	Article de revue
Auteur	Akshita Sharma
Auteur	Ahmad Joshkon
Auteur	Aymen Ladjimi
Auteur	Waël Traboulsi
Auteur	Richard Bachelier
Auteur	Stéphane Robert
Auteur	Alexandrine Foucault-Bertaud
Auteur	Aurélie S. Leroyer
Auteur	Nathalie Bardin
Auteur	Indumathi Somasundaram
Auteur	Marcel Blot-Chabaud
Résumé	Background: Triple Negative Breast Cancers (TNBC) are the most aggressive breast cancers and lead to poor prognoses. This is due to a high resistance to therapies, mainly because of the presence of Cancer Stem Cells (CSCs). Plasticity, a feature of CSCs, is acquired through the Epithelial to Mesenchymal Transition (EMT), a process that has been recently shown to be regulated by a key molecule, CD146. Of interest, CD146 is over-expressed in TNBC. Methods: The MDA-MB-231 TNBC cell line was used as a model to study the role of CD146 and its secreted soluble form (sCD146) in the development and dissemination of TNBC using in vitro and in vivo studies. Results: High expression of CD146 in a majority of MDA-MB-231 cells leads to an increased secretion of sCD146 that up-regulates the expression of EMT and CSC markers on the cells. These effects can be blocked with a specific anti-sCD146 antibody, M2J-1 mAb. M2J-1 mAb was able to reduce tumour development and dissemination in a model of cells xenografted in nude mice and an experimental model of metastasis, respectively, in part through its effects on CSC. Conclusion: We propose that M2J-1 mAb could be used as an additional therapeutic approach to fight TNBC.
Date	2022/1
Langue	en
Catalogue de bibl.	www.mdpi.com
URL	https://www.mdpi.com/1422-0067/23/2/974
Consulté le	17/01/2022 à 10:38:09
Autorisations	http://creativecommons.org/licenses/by/3.0/
Extra	Number: 2 Publisher: Multidisciplinary Digital Publishing Institute
Volume	23

Pages	974
Publication	International Journal of Molecular Sciences
DOI	10.3390/ijms23020974
Numéro	2
Date d'ajout	17/01/2022 à 10:38:09
Modifié le	17/01/2022 à 10:38:09

- **Marqueurs :**
  - CD146
  - treatment
  - triple negative breast cancer

Pièces jointes

  - Full Text PDF
  - Snapshot

- # The Interaction of Platelets with Colorectal Cancer Cells Inhibits Tumor Growth but Promotes Metastasis

Type	Article de revue
Auteur	Léa Plantureux
Auteur	Diane Mège
Auteur	Lydie Crescence
Auteur	Estelle Carminita
Auteur	Stéphane Robert
Auteur	Sylvie Cointe
Auteur	Nicolas Brouilly
Auteur	Walid Ezzedine
Auteur	Françoise Dignat-George
Auteur	Christophe Dubois
Auteur	Laurence Panicot-Dubois
Résumé	Platelets promote metastasis, however, their role in tumor growth remains controversial. Here, we investigated the effect of platelet interactions with colorectal tumor cells. Platelets extravasated into the tumor microenvironment and interacted with tumor cells in a cadherin-6-dependent manner. The interaction induced platelet spreading, release of their granule content, and the generation of three types of microparticles (iMP) that expressed platelet markers, tumor markers, or both. The presence of iMPs was confirmed in colorectal cancer tissue specimens. Platelets significantly reduced tumor growth and increased intratumoral macrophages. This was mediated by iMP recruitment of macrophages via the chemoattractants RANTES, MIF, CCL2, and CXCL12 and activation of their tumor cell killing capacity through

IFNγ and IL4, which led to cell-cycle arrest of tumor cells in a p21-dependent manner. In contrast, in the bloodstream, iMPs activated endothelial cells and platelets and induced epithelial-to-mesenchymal transition of tumor cells, promoting metastasis. Altogether, these results indicate that depending on the environment, local or bloodstream, the consequences of the interactions between platelets and a tumor may promote or prevent cancer progression. SIGNIFICANCE: Tumor cell interaction with platelets produces chimeric extracellular vesicles that suppress primary tumor growth by activating tumor-eliminating macrophages, while promoting metastasis through EMT and endothelial activation.

Date	Jan 15, 2020
Langue	eng
Catalogue de bibl.	PubMed
Extra	Number: 2
Volume	80
Pages	291-303
Publication	Cancer Research
DOI	10.1158/0008-5472.CAN-19-1181
Numéro	2
Abrév. de revue	Cancer Res.
ISSN	1538-7445
Date d'ajout	13/12/2021 à 10:36:31
Modifié le	13/12/2021 à 10:36:31

- **Pièces jointes**
  - PubMed entry