Sickle Cell Disease, from a genetic red cell disease to a clinical inflammatory disease

Caroline le Van Kim
UMR_S1134 Biologie Intégrée du Globule Rouge

GLOBAL SICKLE CELL DISEASE NETWORK

4th Global Congress on Sickle Cell Disease

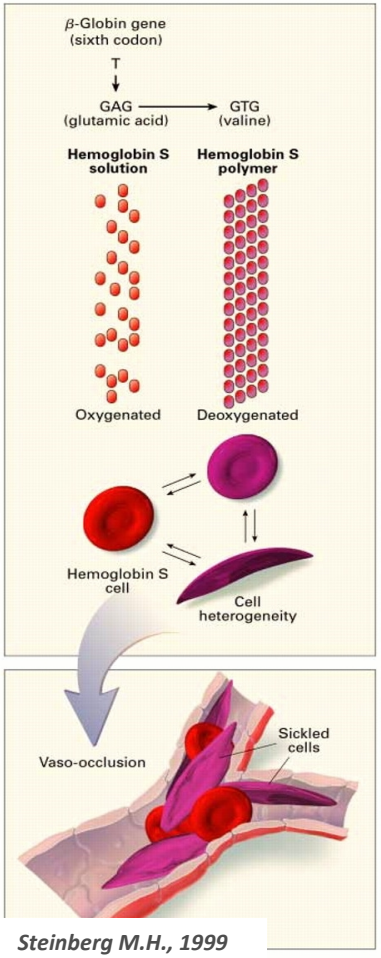
In memory of Paul Frenette

Université Paris Cité

Inserm

HbS : a unique mutation but a polysystemic disease

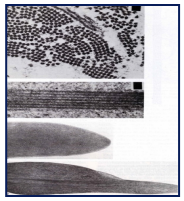
p.Val6Glu → HbS



Steinberg M.H., 1999

HbS polymerisation

Sickled Red Blood Cells



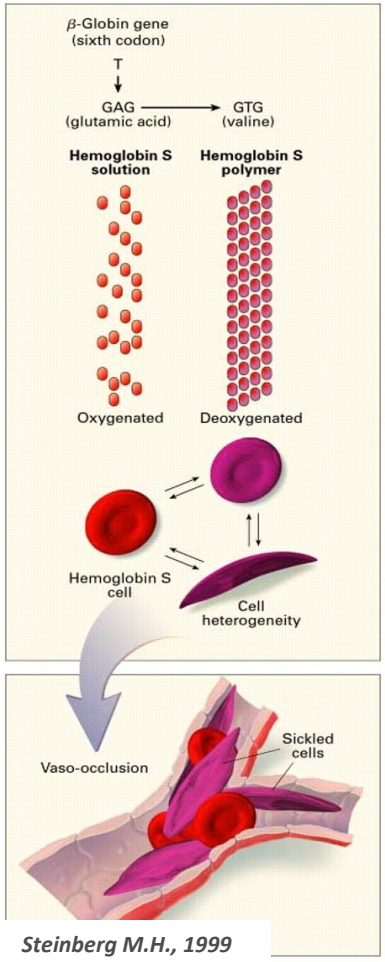
Vasooccclusion

clinical episodes of severe ischemic pain due to the lack of blood flow

- Acute crisis
- dactilytis
- Acute chest syndrome
- splenic sequestration

SCD : much more complex

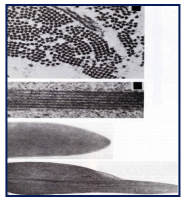
p.Val6Glu → HbS



Steinberg M.H., 1999

Delay of the HbS polymerization and the appearance of crisis

Sickled Red Blood Cells



at least two main triggers in the occurrence of VOC

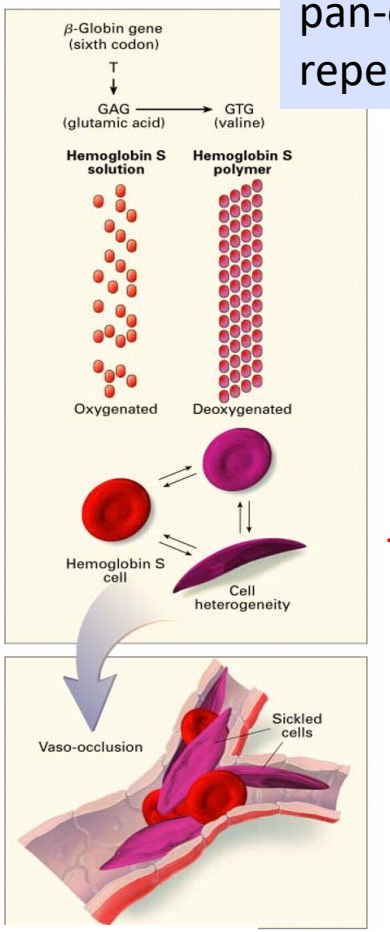
Vasooccclusion

- Acute crisis
- dactilytis
- Acute chest syndrome
- splenic sequestration

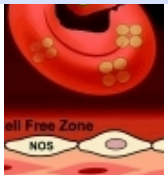
HbS : a unique mutation but a polysystemic disease

p.Val6Glu —

Polymerization of deoxy Hb alters RBCs properties and VO processes =➔ pan-cellular activation ==➔ hemolysis, vaso-occlusion, ischemia-reperfusion =➔ severe complications of the disease.



Steinberg M.H., 1999



Hémolysis

Abnormal adhesion to va

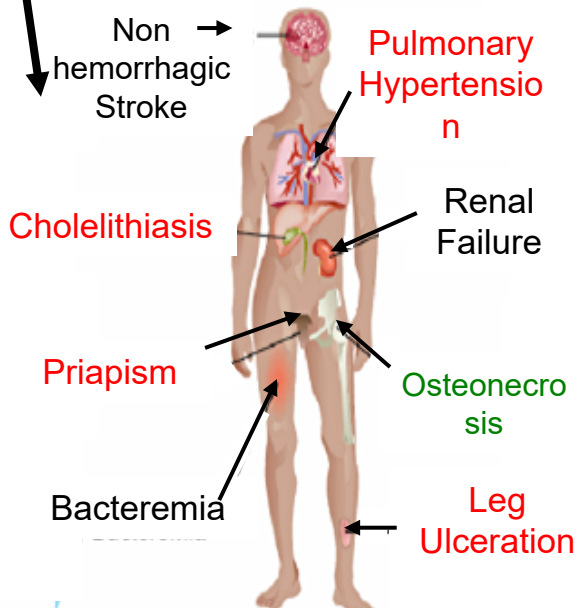
two main triggers

Vasoocclusi

Ischemia-reperfusion

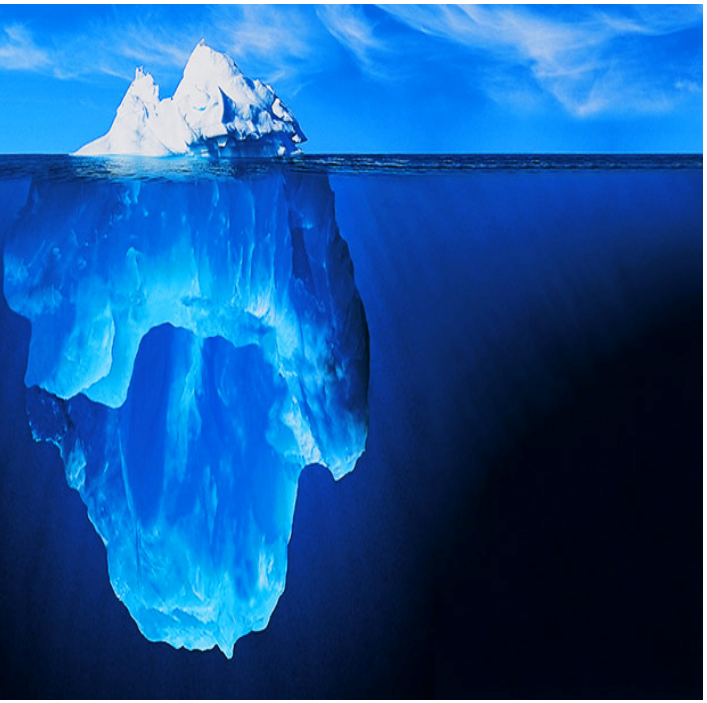
destruction

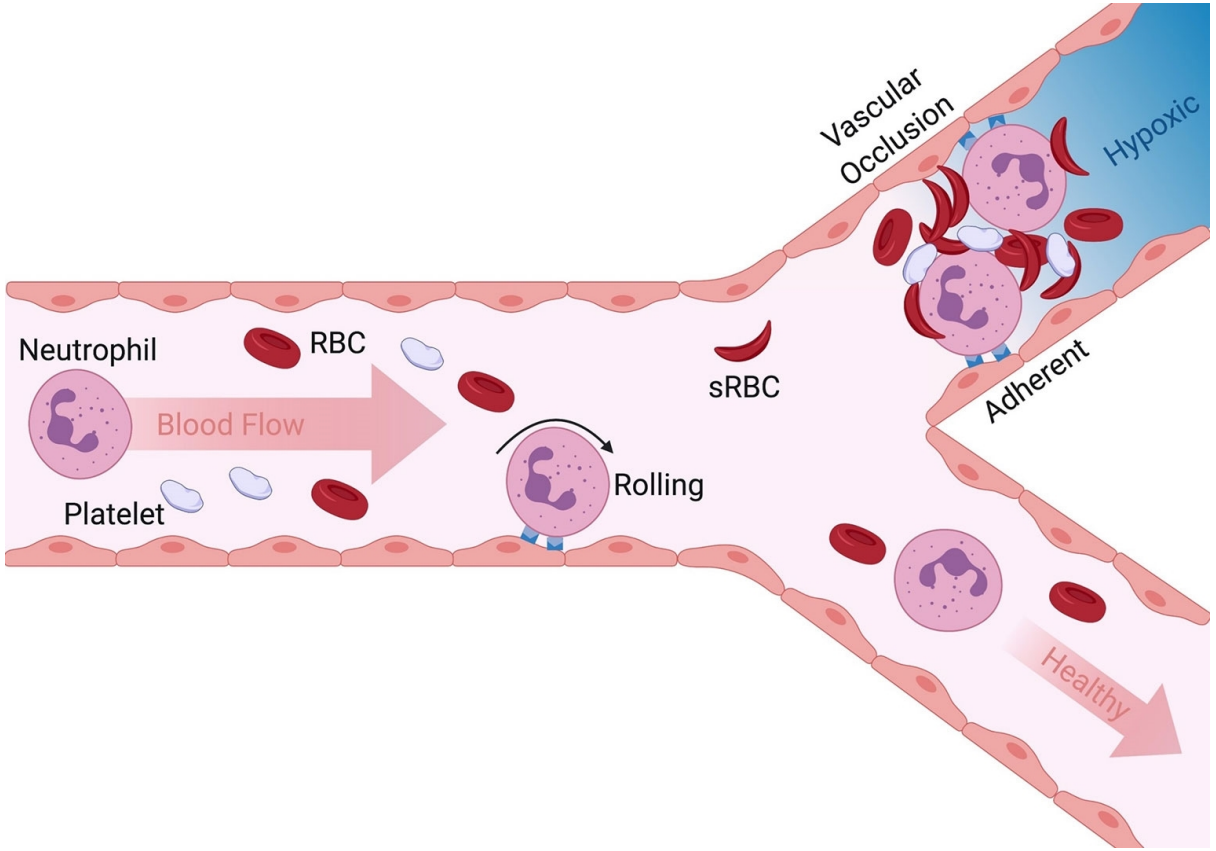
Vicious circle



The vaso-occlusion paradigm: a multistep mechanism and an inflammatory process

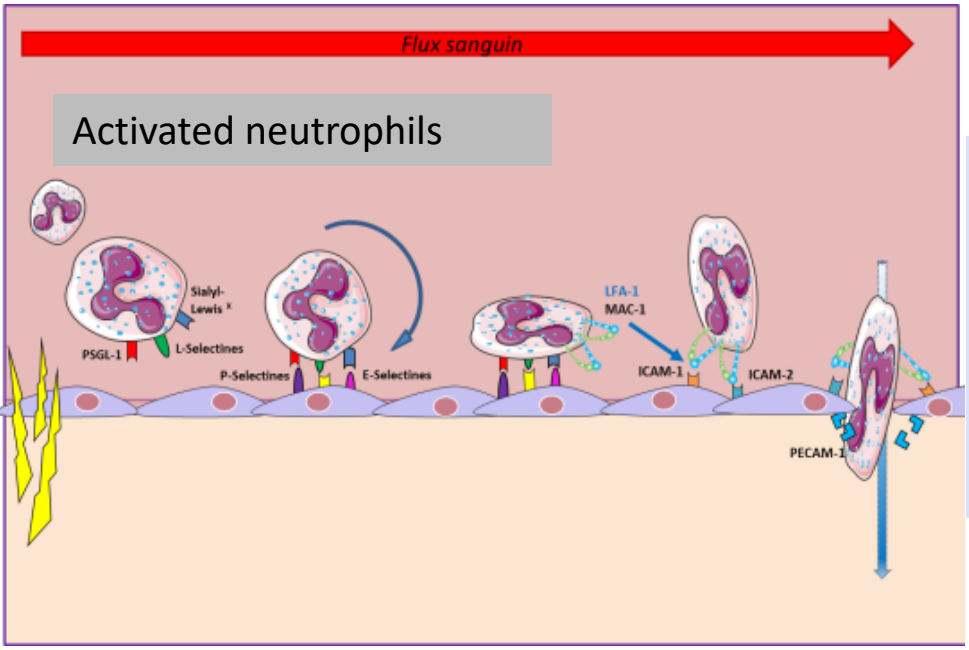
a cascade of interactions between neutrophils, red blood cells, platelets and the activated endothelium





adapted from Frenette et al, 2016

Neutrophil defects

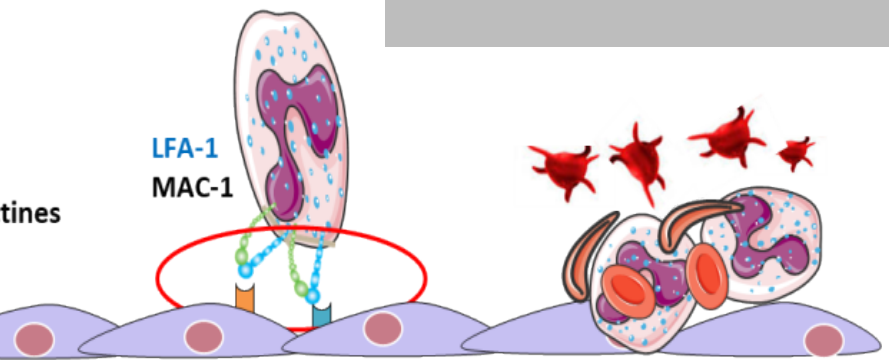


Hyperleukocytosis in patients at steady state associated with: **Origin** ? ?

- **frequent VOC** *(Platt NEJM 94, Miller NEJM 00)*
- **ACS** *(Castro Blood 94, Miller NEJM 00)*
- **Stroke** (Ohene-Frempong Blood 98, Miller NEJM 00)
- **Early mortality** *(Platt NEJM 94, Miller NEJM 00, Gardner Blood 2016)*

Aged neutrophils

Agregates RBCs/neutrophils/platelets

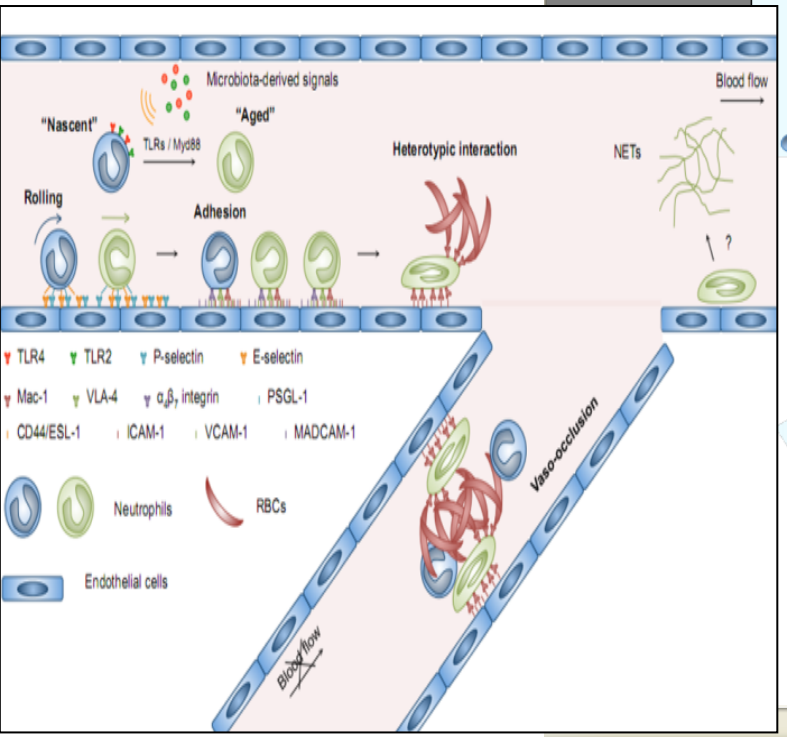


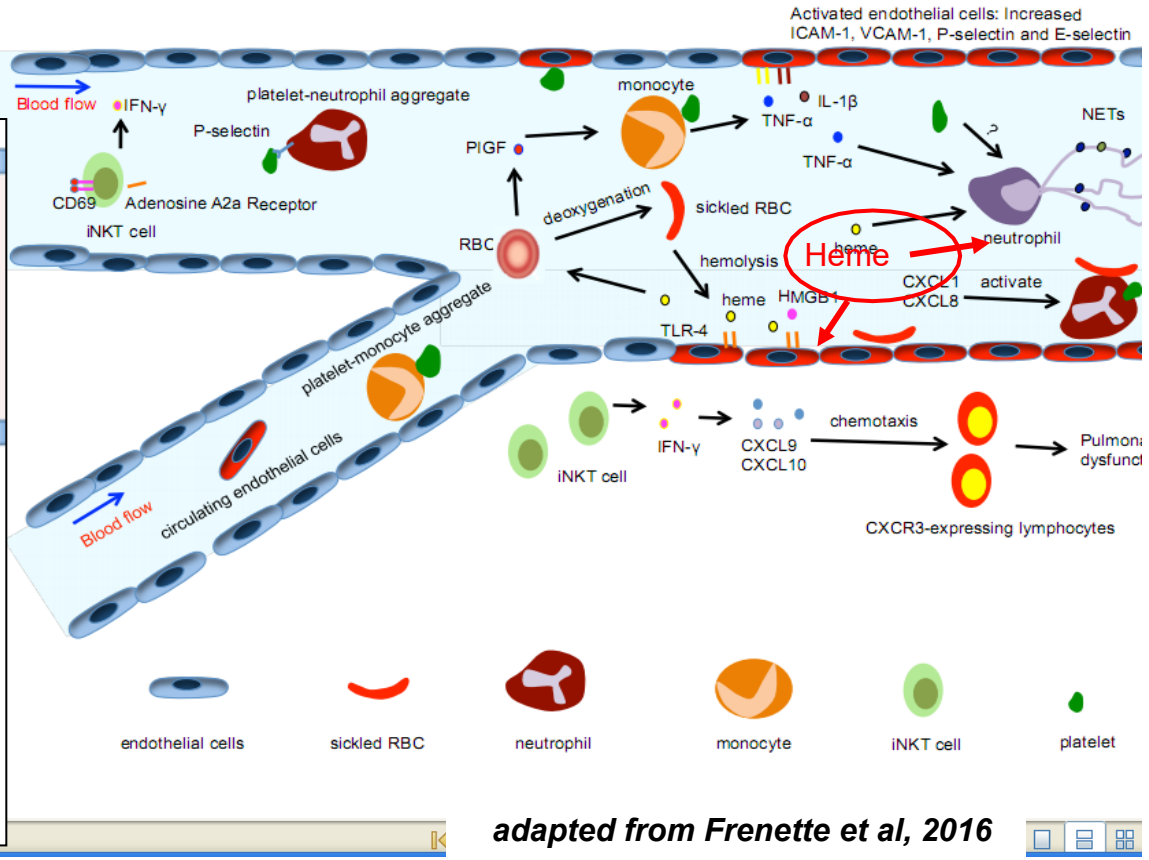
Due to ?

- Free heme
- Hypoxia
- Shear stress
- Ischemia Reperfusion

Vaso-occlusion: a multistep mechanism

- Endothelium and neutrophils activation by free heme
- Captured neutrophils become activated during interaction with endothelium at sites of inflamed microvasculature
- increased adhesion of activated and aged neutrophils
- Aggregates neutrophils/red cells and neutrophils/platelets =➔ rapid occlusion of the microvasculature





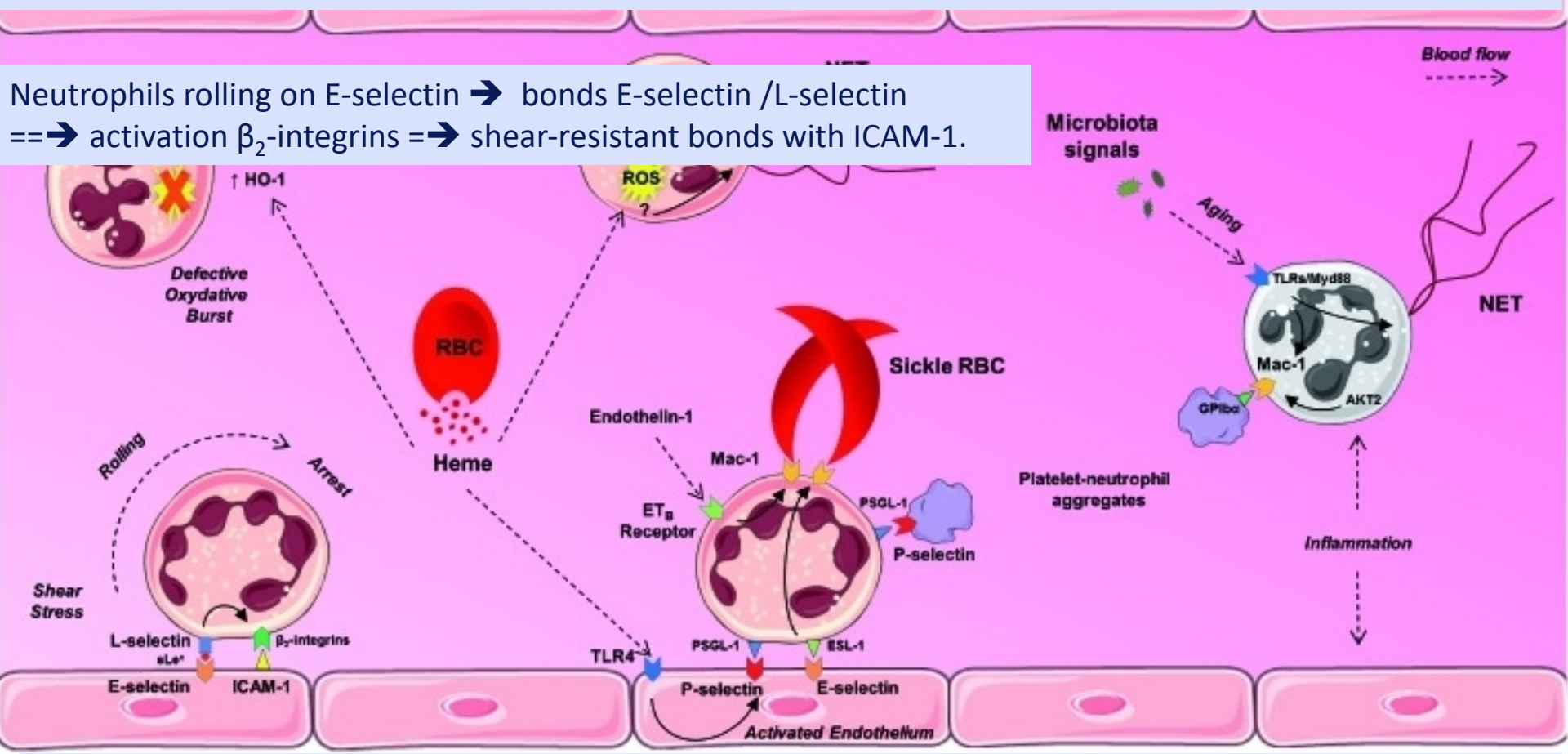
adapted from Frenette et al, 2016

Alali S et al, Haematologica, 2020

Neutrophils in SCD

Neutrophil adhesion : PSGL-1 and endothelial P-selectin interactions upregulated by heme. E-selectin induces a secondary wave of activating signals, =➔ clustering of Mac-1 on the leading edge of adherent neutrophils, allowing for the capture of sickle RBC.

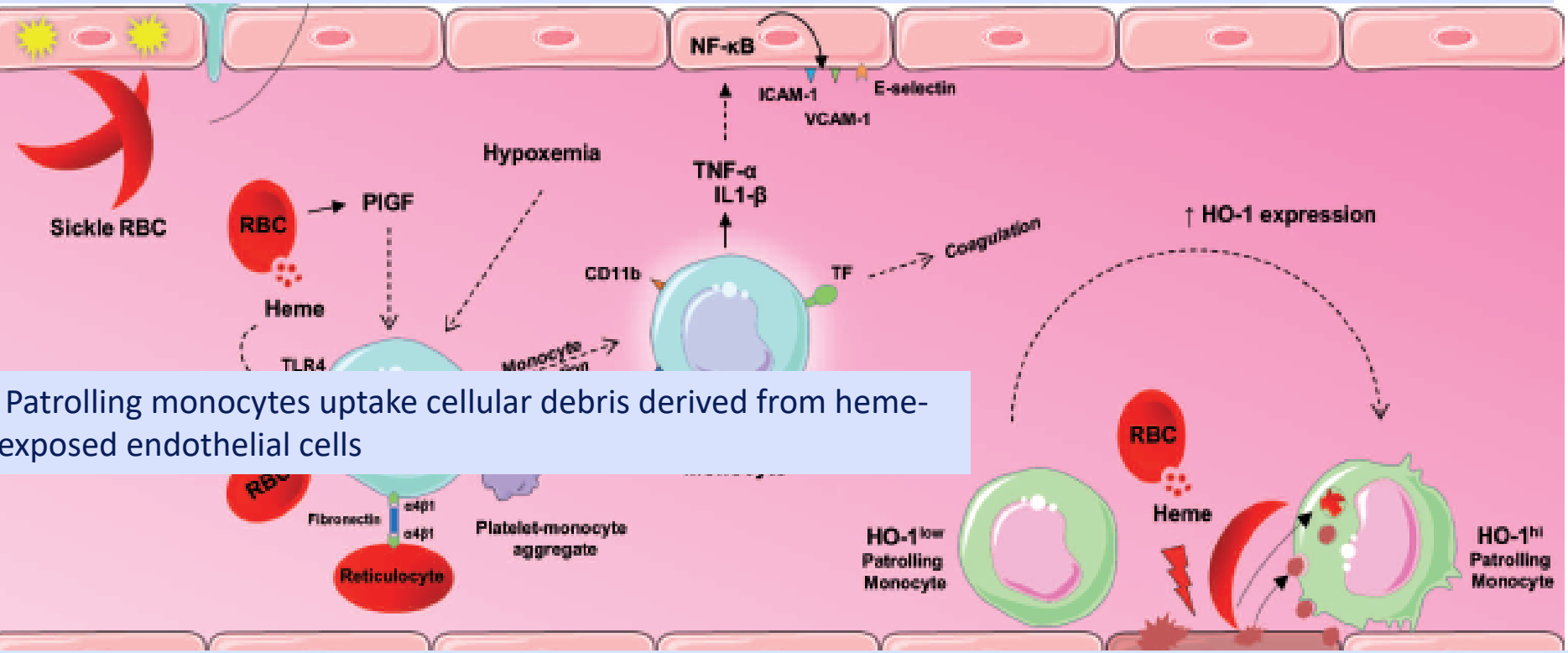
Neutrophils rolling on E-selectin ➔ bonds E-selectin /L-selectin ==➔ activation β_2-integrins =➔ shear-resistant bonds with ICAM-1.



Activated platelets =➔ platelet-neutrophil aggregates, P-selectin/ PSGL-1 .
Neutrophil aging promoted by the microbiota correlated with Mac-1 expression and NET formation.

Monocytes in SCD

Activated monocytes ==➔ activate endothelial cells through the NFκB pathway



Patrolling monocytes uptake cellular debris derived from heme-exposed endothelial cells

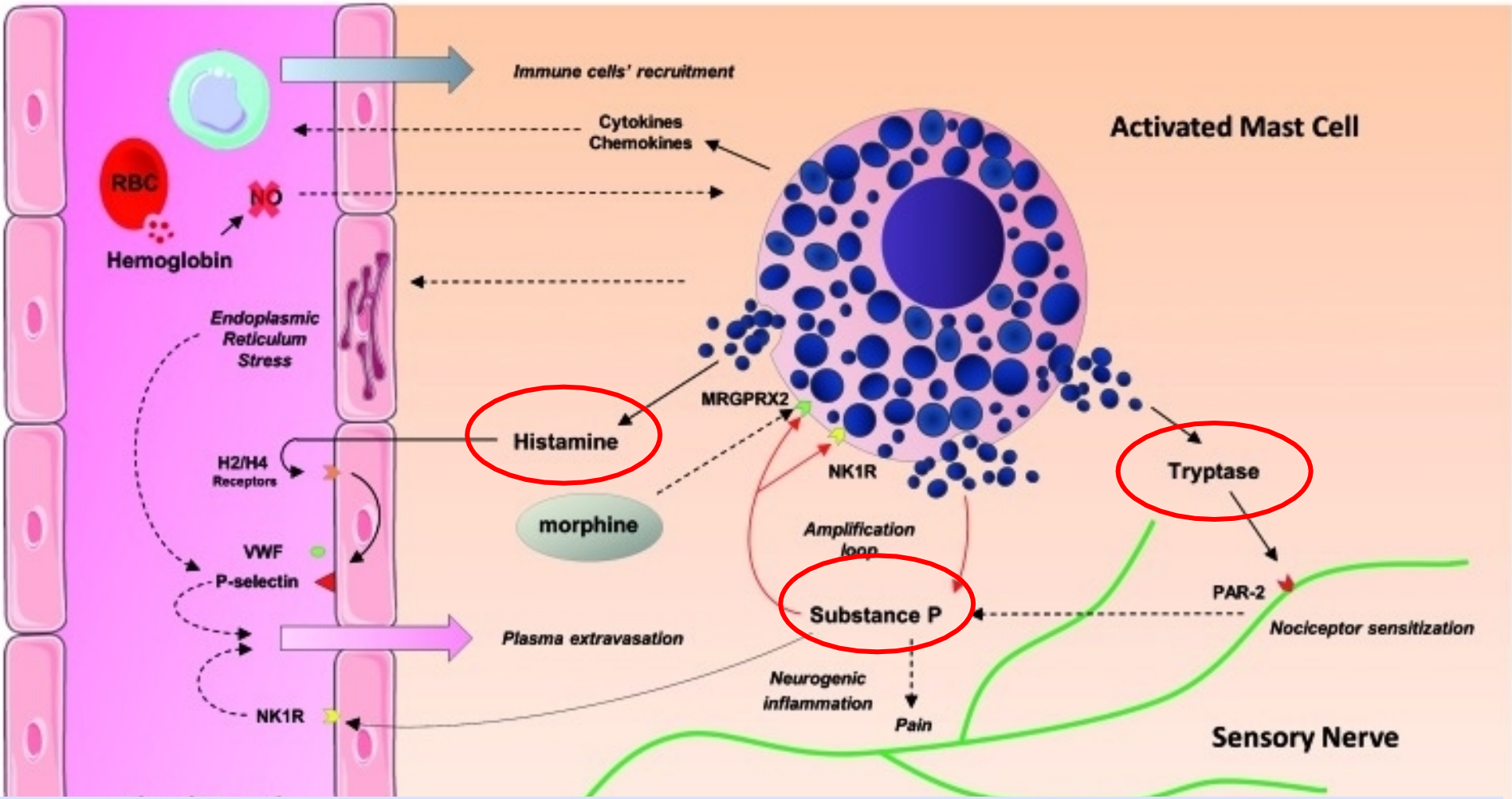
Interaction sickle RBC with endothelial cells=➔ oxidant stress=➔ increased trans-endothelial migration and activation of monocytes=➔ platelet-monocyte aggregates and RBC/retic-monocyte interactions

Figure 1. Monocytes in sickle cell disease. Interaction of sickle red blood cells (RBC) with endothelial cells enhances cellular oxidant stress, resulting in increased

Alali S et al, haematologica, 2020

Mast cells in SCD

Alali S et al, haematologica, 2020



Histamine stimulates endothelial H2 and H4 receptors and expression of P-selectin.

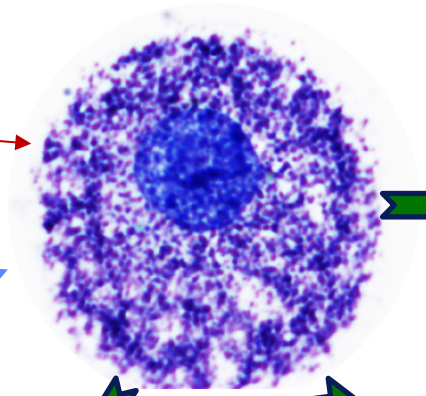
Tryptase activates protease-activated receptor 2 on peripheral nerve endings, contributing to nociceptor sensitization and stimulating the release of **substance P** ➔ promotes neurogenic inflammation

Mastocyte dysfunction

Morphine =➔ mast cells degranulation

➢ Deleterious effect of high dose morphine?

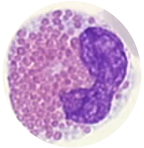
Heme
Chelation of NO



Stimulation of nociceptive nerve endings

Tryptase

+



Inflammation

+

Histamine

Substance P

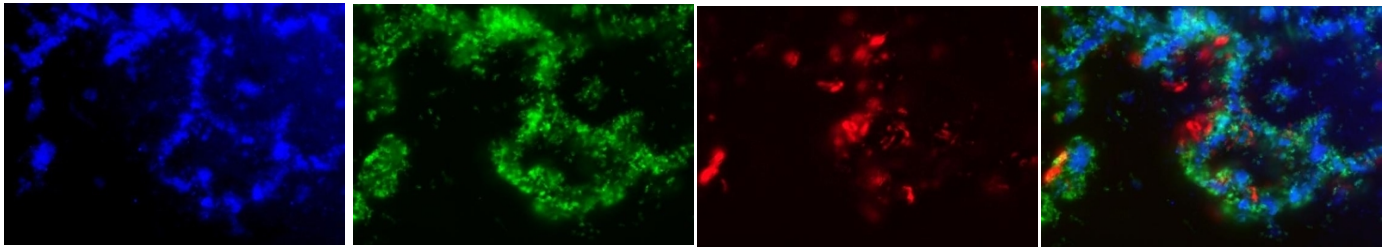
Endothelial cells:
↗ *vWF*
↗ *P-selectins*

Neurotransmitter:
Neurogenic inflammation
Nociceptive signal

Hemolysis may contribute to Mast activation because it is responsible for NO depletion, known to activate Mast cells.

Platelets dysfunction and prothrombotic state in SCD

Aggregates in human SS blood samples samples: flow adhesion experiments on endothelial cells

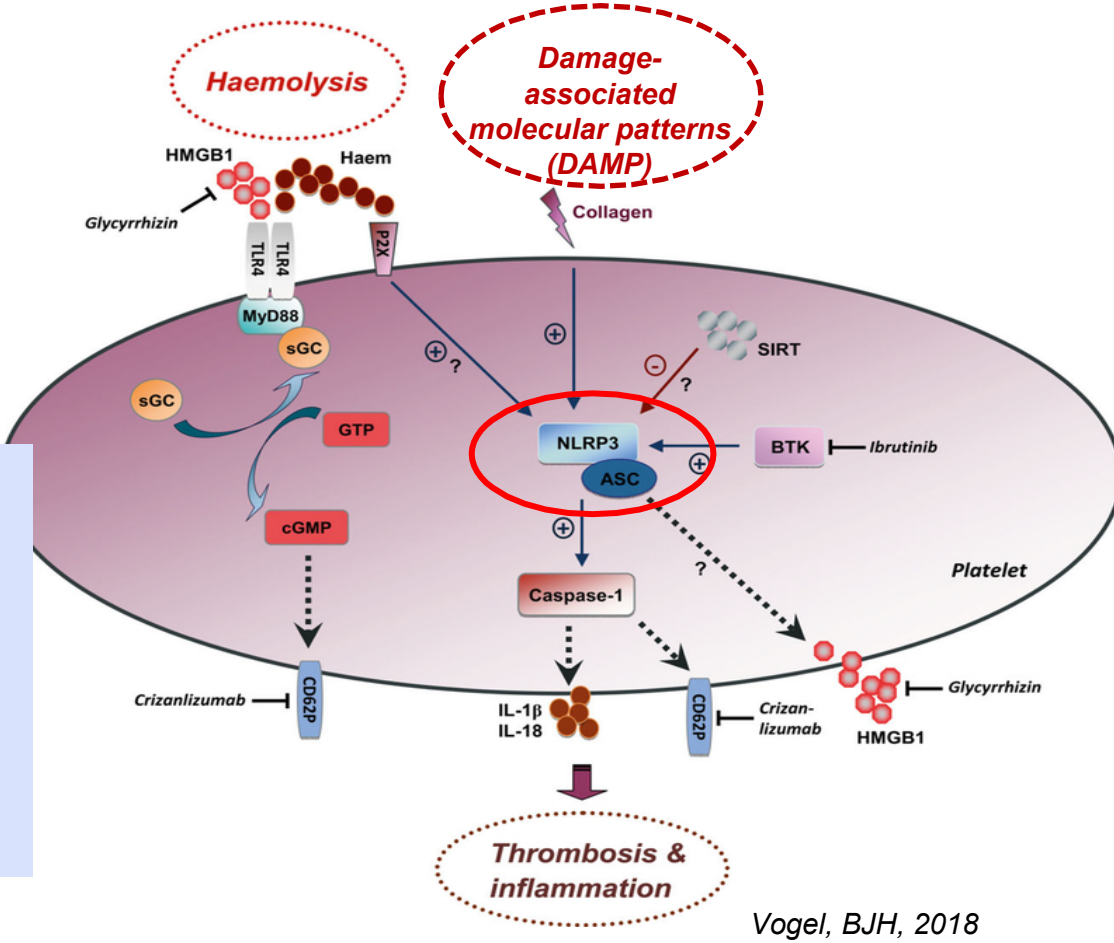


Anti-CD41b (Platelets) | Anti- CD16 (Neutrophils) | Anti-GPA (Erythrocytes) | Merge

Platelets activation:

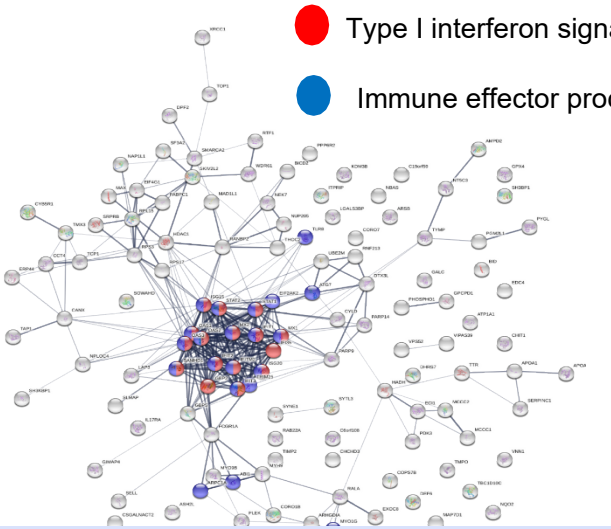
- At basal state and +++ during VOC
- Correlation with risk of ACS and PH
- Cellular adhesion
- Tissular micro thrombi

- Participation in leukocytes/platelet/RBC aggregates
- Liver biopsies during acute pain crises =➔sickled RBCs in association with platelet masses
- **NLRP3 inflammasome** a critical inflammatory mechanism ==➔ platelet activation and aggregation.



Vogel, BJH, 2018

Neutrophils and activation of Interferon-1 signaling pathway in SCD



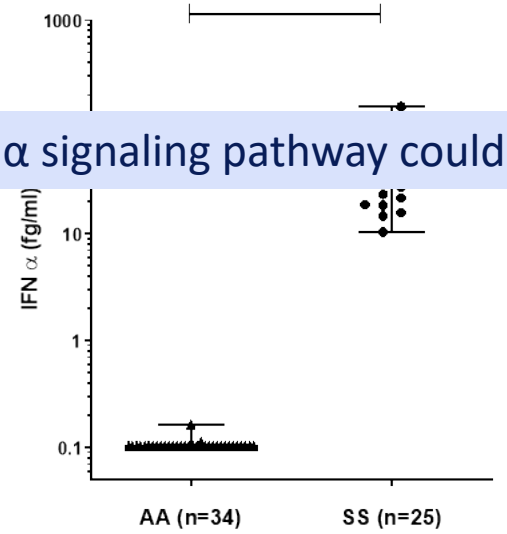
Hermand et al, Haematologica, 2020

→ Quantitative proteome of neutrophils from SS pediatric patients

→ Transcriptomic analyses of IFN Signaling Proteins

STAT1, STAT2, OAS 1,2,3, IFIT1, IFIT2, IFIT 3, ISG15, ISG20, GBP2, IFI35, MX1 et MX2, TLR8
Between 1.5 and 100 fold >>> in neutrophils from SS patients

High-level expression of ISP in neutrophils and of plasmatic IFN from SS patients suggesting an abnormal activation that could be important in developing new anti-inflammatory therapies.



Dysfunction of the IFNα signaling pathway could play a role in the pathogenesis of SCD.

Activation of IFN-1 signaling pathway: consequences?

Type I IFN Is Necessary and Sufficient for Inflammation-Induced Red Blood Cell Alloimmunization in Mice

David R. Gibb, Jingchun Liu, Prabitha Natarajan, Manjula Santhanakrishnan, David J. Madrid, Stephanie C. Eisenbarth, James C. Zimring, Akiko Iwasaki and Jeanne E. Hendrickson

Type 1 Interferon Gene Signature Promotes RBC Alloimmunization in a Lupus Mouse Model

June Young Lee[1†], Emaan Madany[1†], Najwa El Kadi[1], Sumaarg Pandya[1], Kessandra Ng[1], Michifumi Yamashita[1], Caroline A. Jefferies[2] and David R. Gibb[1,3]*

[1] Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States,
[2] Department of Internal Medicine, Division of Rheumatology, Cedars-Sinai Medical Center, Los Angeles, CA, United States,
[3] Division of Transfusion Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States

- Does IFN-1 signaling pathways participate to allo-immunization in SCD?

COVID-19 and IFN-1 and SCA patients

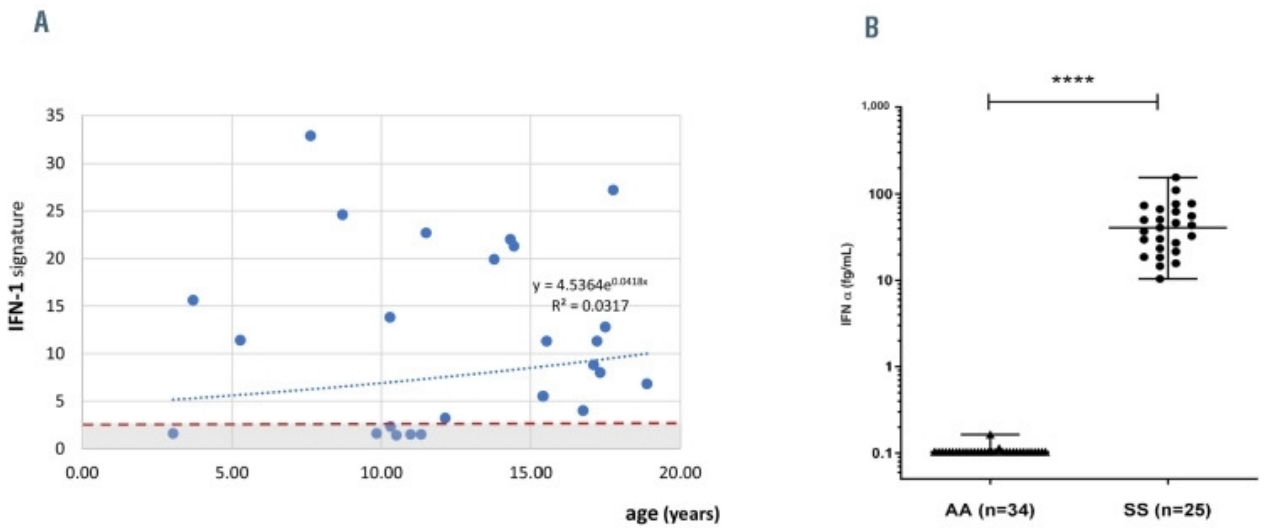
Low incidence of COVID-19 severe complications in a large cohort of children with sickle cell disease: a protective role for basal interferon-1 activation? Brousse V, Haematologica. 2021

SCD and COVID:

- → Fear of severe forms (pulmonary+++)
- → Observation at the end of the first wave: few severe forms
- → **Is that the SCD children are not prone to severe infection like their healthy peers or less exposed ?**
- → At Robert Debré hospital in Paris: very low morbidity(almost zero) despite a seroprevalence of 18% at September, 2020 (n=211)

➢ In SCD children : protective effect of basal activation of IFN-1 signaling pathway on the severe COVID-19?

==➔ High IFN-1 signature in pediatric patients with SCD, including asymptomatic COVID+



(A) Interferon-I (IFN-I) signature in 25 SCD patients according to age. IFN-I signature was considered increased above a score of 2.3.
(B) Basal IFNα concentration in plasma from healthy donors (n=34) and SS patients (n=25)

Therapeutics targeting innate immune cells

Alali S et al, haematologica, 2020

Therapeutic agent	Targeted innate immune cells	Mechanism of action	Study ID #	Phase
Hydroxyurea	Neutrophils, eosinophils, monocytes, NK cells, platelets	Multimodal mechanism including myelosuppression	FDA-approved	III
Crizanlizumab (SEG101)	Neutrophils, platelets	P-selectin inhibitor (monoclonal antibody)	NCT03814746 FDA-approved	III
Rivipansel (GMI-1070)	Neutrophils, platelets	Pan-selectin inhibitor	NCT02187003	III
Sevuparin	Neutrophils, platelets	Multimodal mechanism including P- and L-selectin inhibition	NCT02515838	II
IVIG	Neutrophils	Inhibits neutrophil adhesion and RBC-neutrophil interactions	NCT01757418	II
NKTT120	iNKT cells	iNKT cell depletion (monoclonal antibody)	NCT01783691	I
Ticagrelor	Platelets	ADP receptor antagonist	NCT03615924	III

ID #: identification number; NK cells: natural killer cells; FDA: US Food and Drug Administration; IVIG: intravenous immunoglobulin; RBC: red blood cell; iNKT cells: invarian
ural killer T cells; ADP: adenosine diphosphate.

A randomized, placebo-controlled, double-blind trial of canakinumab in children and young adults with sickle cell anemia

David C. Rees et al, Blood, 2022

- IL-1beta blockade by **canakinumab** (targeting the effect of inflammasome) in patients would reduce markers of inflammation and clinical disease.

=➔ Multicenter phase 2a study, patients aged 8 to 20 years, history of acute pain episodes, and elevated C-reactive protein >1.0 mg/L, received 6 monthly treatments with 300 mg subcutaneous canakinumab or placebo. 1:1

- Although the primary objective (reduction of pain) was not met, patients treated with canakinumab **had reductions in markers of inflammation, occurrence of SCA-related adverse events, and number and duration of hospitalizations .**

- Inflammation associated with SCD can be reduced by selective IL-1beta blockade by canakinumab with potential for therapeutic benefits.

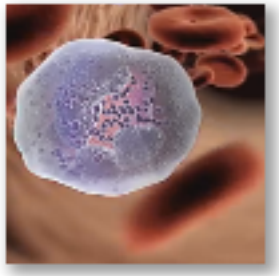
  This trial was registered at www.clinicaltrials.gov as #NCT02961218.

First taking home messages

- Activation of many innate immune actors in the basal state in SCD
- Chronic inflammatory disease
- Peripheral origin (free heme, shear stress, hypoxia-reperfusion...) but also possibly CENTRAL (abnormal engagement of the myeloid pathway ??)
- Most often deleterious...except in the context of a viral pandemic?

Persistence of Chronic Inflammation Despite Years of Transfusion Program in SCD Patients:

Changing Red Blood Cells Is Not Sufficient to Treat Sickle Cell Disease ??



Courtesy of Bérengère KOEHL, MD, PhD (ungoing simultaneous session)

Sickle Cell Disease Center, Hematology unit

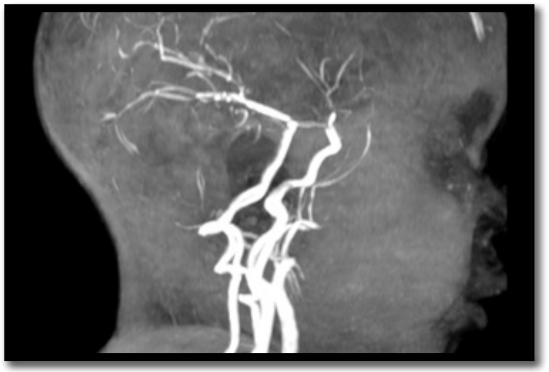
Robert Debré Hospital

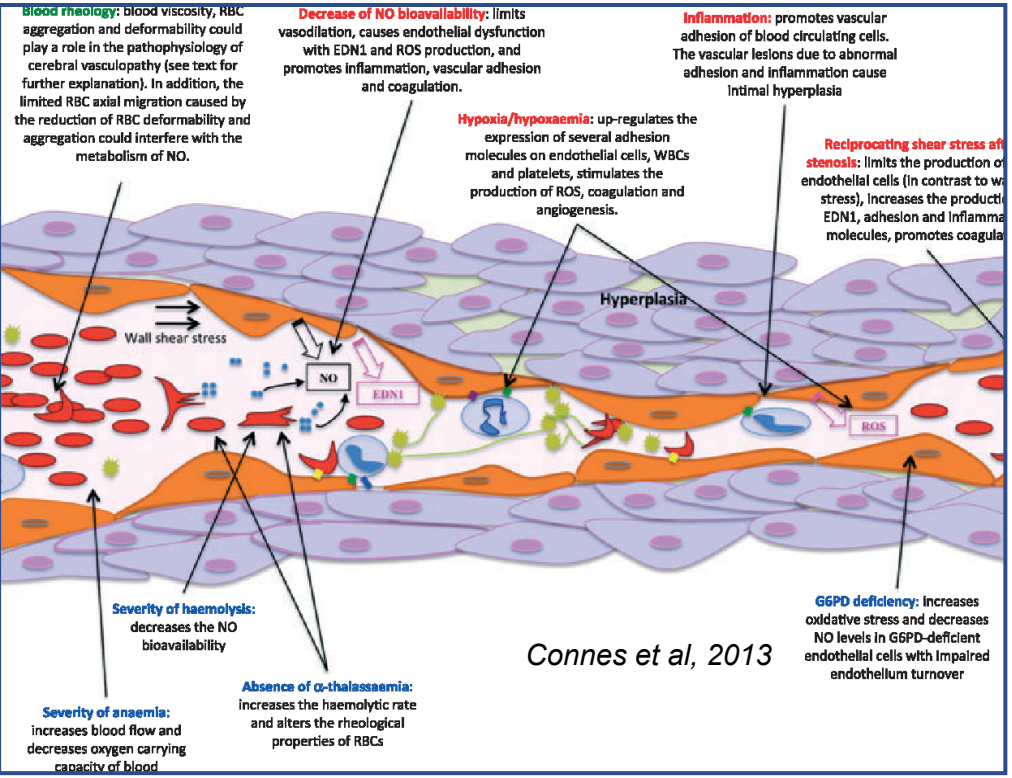
INSERM UMR_S1134 Red Blood Cell Biology

Paris, France

Cerebral Vasculopathy in children with SCD

- Progressive circumferential stenosis of the cerebral arteries of the Willis circle
- Progressive occlusion → ischemic stroke in 75% of cases





Complex pathophysiology of CV:

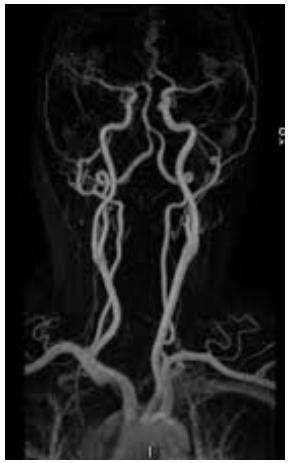
- Blood rheology, blood cells aggregation,
- shear stress
- Decrease of NO availability
- Local hypoxia
- Inflammation

Preventive and curative treatment for Cerebral Vasculopathy in SCD children

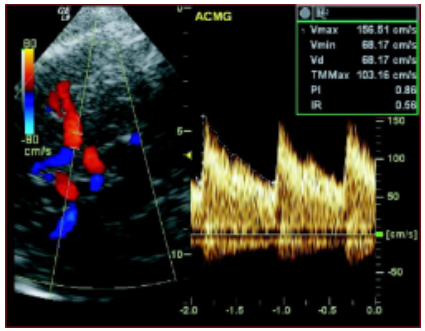
The NEW ENGLAND JOURNAL of MEDICINE 1998 Jul 2;339(1):5-11.

Prevention of a First Stroke by Transfusions in Children with Sickle Cell Anemia and Abnormal Results on Transcranial Doppler Ultrasonography

Robert J. Adams, M.D., Virgil C. McKie, M.D., Lewis Hsu, M.D., Ph.D., Beatrice Files, M.D., Elliott Vichinsky, M.D., Charles Pegelow, M.D., Miguel Abboud, M.D., Dianne Gallagher, M.S., Abdullah Kutlar, M.D., Fenwick T. Nichols, M.D., Duane R. Bonds, M.D., Donald Brambilla, Ph.D., et al.



- Systematic screening from the age of 18 months: TCD and Angio-RMI
- In case of confirmed cerebral vasculopathy:
  - ➢ Monthly transfusion/exchange transfusion
  - ➢ Objective : Maintaining an HbS rate < 30 %

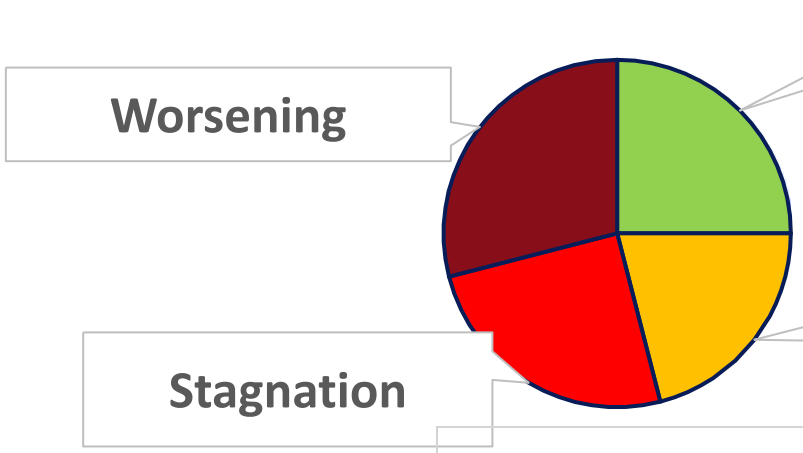


➔ Stroke prevention STOP Study in 1998 =➔ Transfusion programs : reducing by 92% the occurrence of stroke in children with cerebral vasculopathy

Persistance of Cerebral vasculopathy despite Exchange transfusion

High heterogeneity ➔ Evolution of CV in children under chronic exchange transfusion

(Bader-Meunier, Haematologica, 2009)



After about 2 years of chronic transfusions, only a quarter of the patients have normalized their arteries.

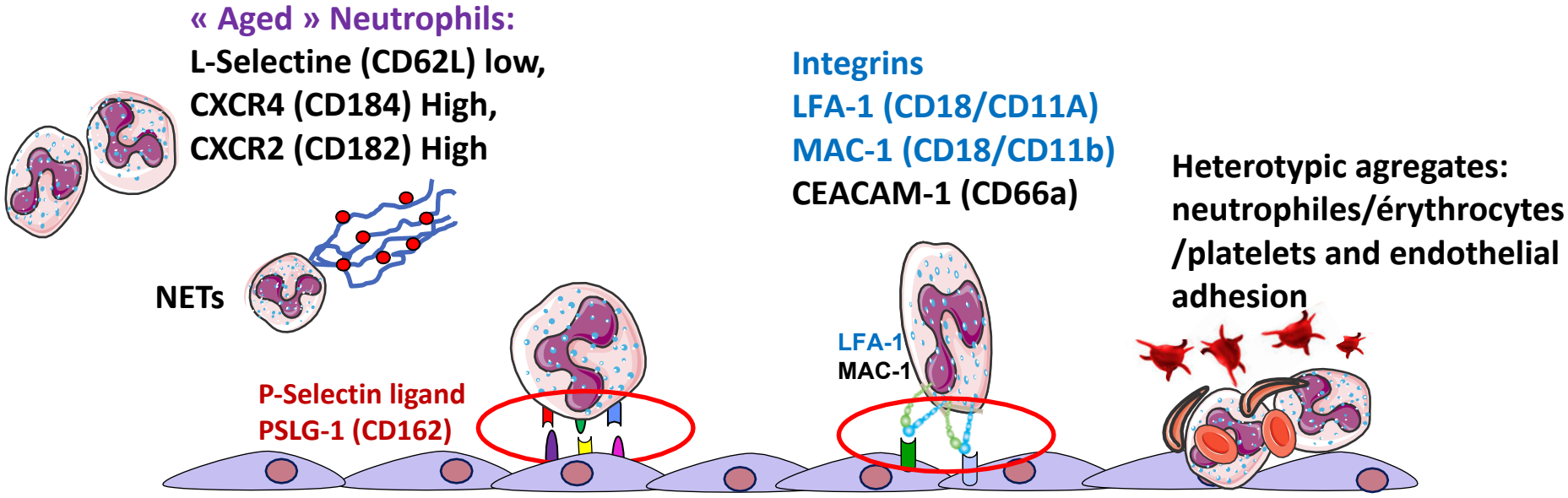
	Improvement	Stagnation	Worsening	Criteria of CV prior to ET
Abnormal TDC in intracranial arteries and normal cerebral RMA	n= 7	n= 5	n= 0	n= 12 (13.6%)
Stenosis of intracranial arteries on RMA	n=10	n=21	n=10	n= 41 (46.6%)
Stenosis of cervical arteries on RMA	n= 1	n=13	n=0	n= 14 (15.9%)
Initial Stroke leading to the diagnosis of stenosis of intracranial arteries on RMA	n=1	n=15	n=5	n=21 (23.9%)
Evolution of the CV under ET program (% of the whole cohort)	n=19 (21.6%)	n=54 (61.4%)	n=15 (17.0%)	n=88 (100%)

Consistent with these observations, 78 % of patients under transfusion program have a stable or worsening vasculopathy since the onset of the program.

Which process is responsible for persistent / evolutive vascular lesions despite the replacement of sickle red blood cells?

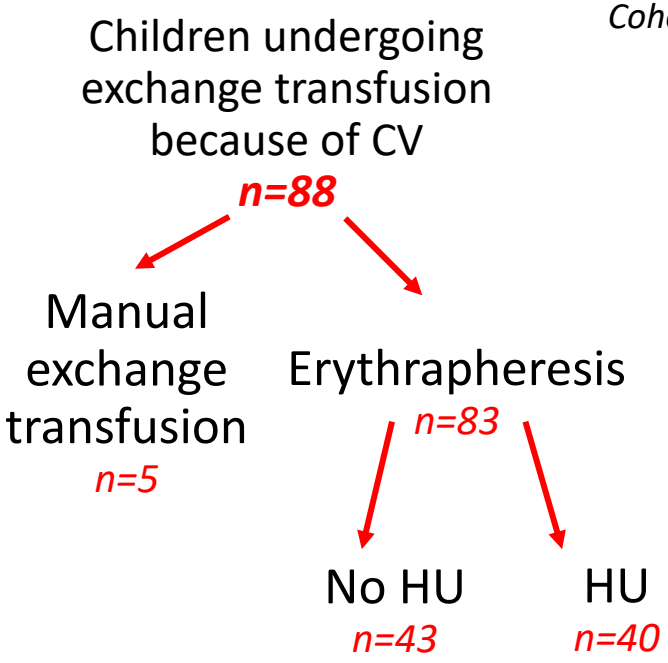
Could the persistence of leukocyte inflammation contribute to the maintenance of
Cerebral vasculopathy despite years of transfusions and the maintenance of an HbS level < 40%?

Objectives



- Impact of transfusion programs on neutrophil counts, ageing, activation and adhesion, compared to non-transfused patients

High leucocytosis persists despite exchange transfusion (ET)



Cohort Robert Debré, Paris Bérengère Koehl

	Erythrapheresis	Manual exchange
n	43	5
Hb level before/during ET program (g/dl)(median (IC)	7.5 (6.9 ; 8.5) / 9.1 (8.6 ; 9.5)****	9.1 (8.8 ; 9.2) / 9.7 (8.9 ; 9.7) *(ns)*
Leukocyte count before/during ET program (G/L) *(median (IC)*	11 800 (9 630 ; 14 500) / 11 365 (9 723 ; 12 378)**	12 580 (12 030 ; 18 780) / 12 010 (8 145 ; 16 040) *(ns)*
Neutrophils count before/during ET program (G/L) *(median (IC)*	4 984 (3 480 ; 6 596) / 6 275 (4 999 ; 7 998) *(ns)*	6 076 (4 896 ; 9 259) / 5 054 (2 765 ; 8 378) *(ns)*
Monocytes count before/during ET program (G/L) *(median (IC)*	1 095 (713 ; 1 493) / 1 373 (977 ; 1 609) *(ns)*	560 (529 ; 591) / 803 (542 ; 992) *(ns)*

- Mean Age: **11.05 yo**
- Mean Duration of the ET program: **4.24 years**

Efficiency of the ET program:

- Mean HbS rate after ET session: **13.4 %**
- Mean HbS rate before the next ET session: **35.1%**

Tolerance of the ET program:

- Mean ferritinaemia: 352 µg/l (35; 1061) without iron chelators

→ Persistence of high neutrophil count and high monocyte count

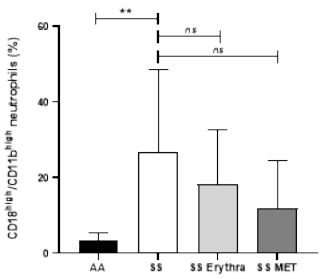
→ Both with erythrapheresis and manual exchange

→ Stable despite 4.24 years of ET program

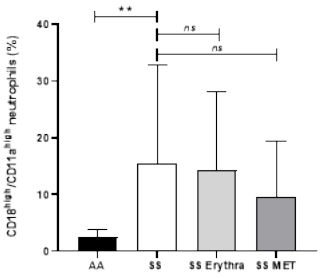
Neutrophils from SCA patients undergoing exchange transfusions have the same pathological phenotype as those from untreated patients

Expression of adhesion molecules and proportion of aged neutrophils by flow cytometry

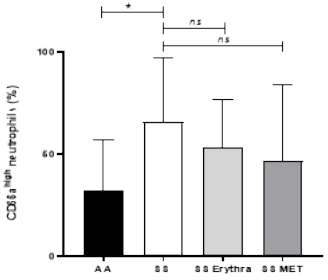
Mac-1 integrin expression



LFA-1 integrin expression



CD66a expression



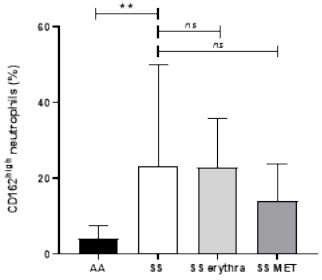
AA healthy controls

SS patients

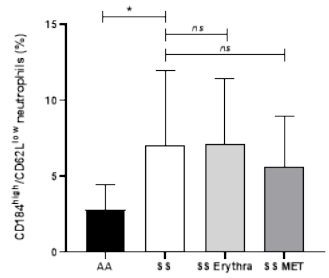
SS patients with erythrapheresis

SS patients with manual exchanges

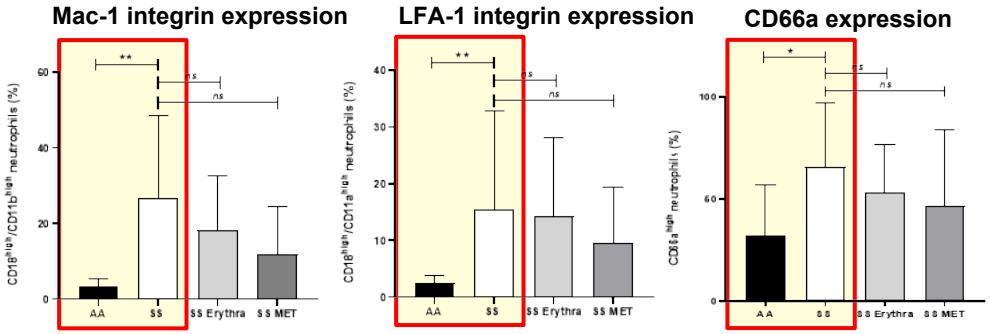
PSLG-1 expression



% Aged neutrophils (CXCR4^{high}/CD62L^{low})



Neutrophils from SCA patients undergoing exchange transfusions have the same pathological phenotype as those from untreated patients

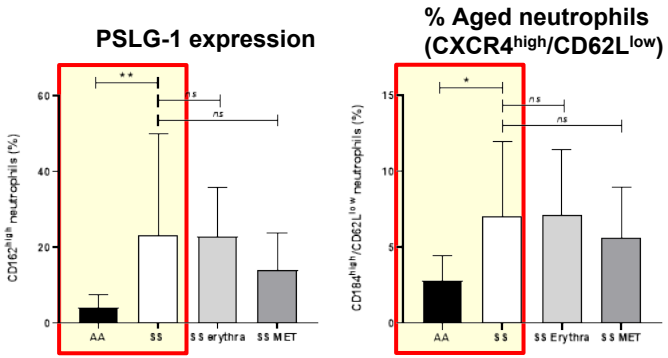


AA healthy controls

SS patients

SS patients with erythrapheresis

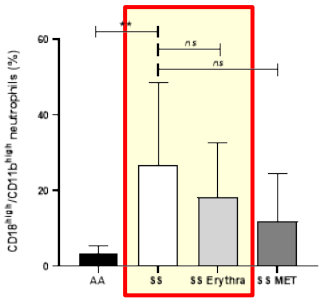
SS patients with manual exchanges



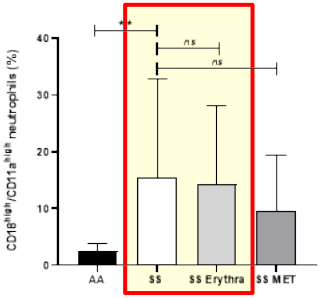
neutrophils from SS patients exhibit high expression of adhesion molecules, integrin Mac-1, LFA-1, CD66a and PSLG-1 and high proportion of circulating aged-neutrophils compared to AA controls.

Neutrophils from SS patients undergoing exchange transfusions have the same pathological phenotype as those from untreated patients

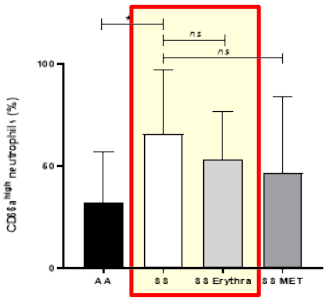
Mac-1 integrin expression



LFA-1 integrin expression



CD66a expression



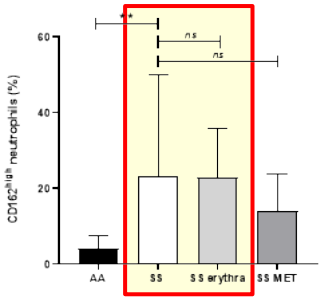
AA healthy controls

SS patients

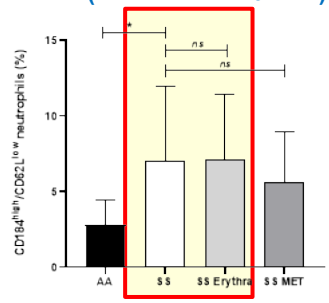
SS patients with erythrapheresis

SS patients with manual exchanges

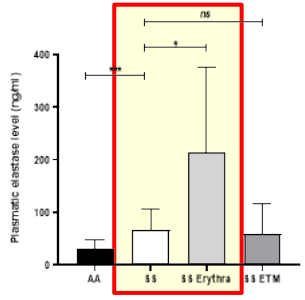
PSLG-1 expression



% Aged neutrophils (CXCR4^{high}/CD62L^{low})

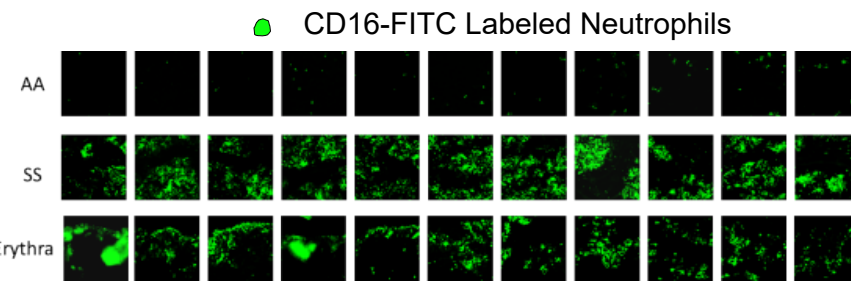


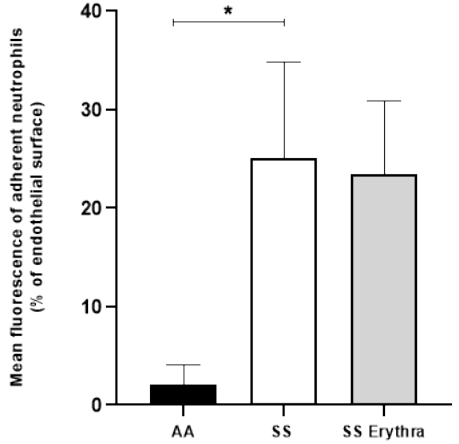
Plasmatic elastase



High level of Netosis (elastase) in SS patients and after erythrapheresis

Functional impact ➔ Neutrophils adhesion on endothelial cells by flow adhesion experiments





% of endothelial surface covered by adherent neutrophils

High level of neutrophils adhesion in non-treated SS patients and under erythrapheresis

Conclusions and Perspectives

- Children with SCA on chronic transfusion sustain chronic inflammation and an abnormal neutrophil phenotype, which may contribute to vascular damage.

- Decreasing the proportion of SS RBC is not sufficient to reverse the quantitative and qualitative defects of neutrophils

- Neutrophil's defects driven by hemolysis or…other origin : hypothesis of central origin ?

Thank you for your attention

INSERM UMR_S 1134
Integrated Biology of Red Blood Cells
Equipe 1- Pathophysiology of RBC
Paris, France

Bérengère Koehl
Abdoul Karim DEMBELE
Patricia HERMAND-TOURNAMILLE
Slim Azouzi
Valentine Brousse
Yves COLIN-ARONOVICZ

Sickle cell Disease Center
Hematology Unit
Robert Debré Hospital
Paris, France

Malika BENKERROU

Etablissement Français du Sang
Emmanuelle LESPRIT
Anne ARNOULT

3P5 proteomic platfom