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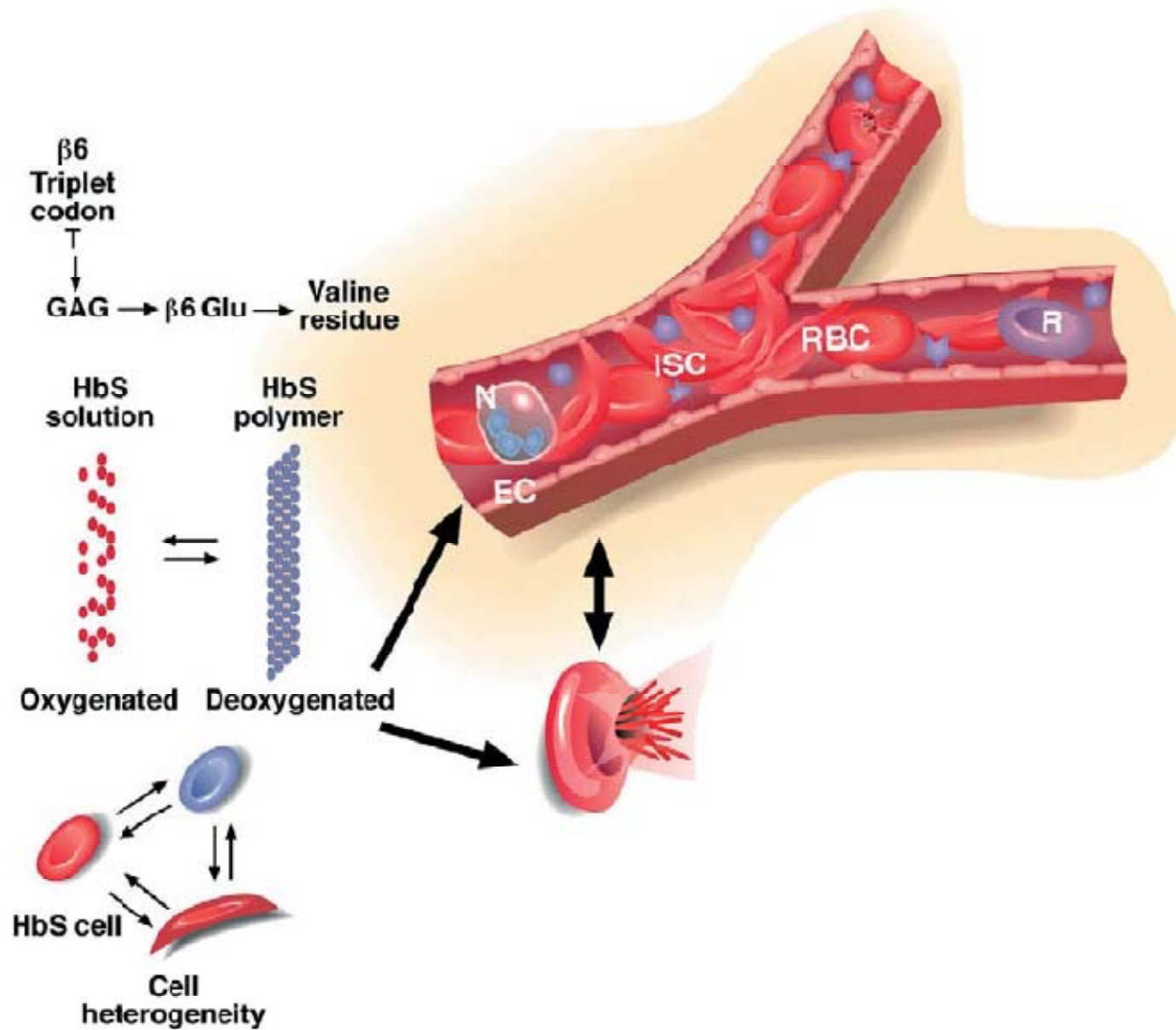
# **INFLUENCE OF HYDROXYUREA TREATMENT ON THE ADHESIVE AND CHEMOTACTIC PROPERTIES AND DEGRANULATION OF EOSINOPHILS IN PATIENTS WITH SICKLE CELL ANEMIA**

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Support:  **FAPESP**

# **INTRODUCTION**

# Vaso-occlusion



# **INFLAMMATION** x **SCD** x **LEUKOCYTES**

- Characterized by a chronic inflammatory state;
- Increased numbers of leukocytes are associated with increased morbidity and mortality in SCD;
- Elevated blood levels of inflammatory mediators and cytokines (PGE<sub>2</sub>, TNF- $\alpha$ , IL-8, IL-6, GM-CSF) both in steady-state and during crisis (Francis et al, 1992, Conran et al, 2007; Lanaro et al, 2009).
- Leukocytes participate significantly in the vaso-occlusive process, appear to have an initiating role (Platt et al, 1994, Frenette, 2002, Turhan, 2002);

# **NEUTROPHILS** x **SCD**


SCD neutrophils appear to exist in a primed or activated state in the circulation and demonstrate significant functional alterations; for example:


- Adhesion molecule expression;
- Adhesivity to ECM proteins, endothelial cell layers and endothelial proteins (Fadlon et al., 1998; Assis et al., 2005; Canalli et al., 2008);
- Gene expressions of IL-8, IFN- $\gamma$ , iNOS, and HO-1 in SCA neutrophils (Lanaro et al., 2009).

Apoptotic processes may be inhibited in sickle cell disease neutrophils (Conran et al, 2007).

# ***EOSINOPHILS***

In response to many stimuli, eosinophils are recruited to the inflammatory focus, which modulates the immune response through a series of mechanisms

Eosinophils  release toxic granule proteins (MCP, EDN and eosinophil peroxidase)

Eosinophils  release IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-18, TGF, chemokines (RANTES, eotaxin-1), PAF and LTC<sub>4</sub>.

**Asthma**

Hogan et al, 2008

# *EOSINOPHILS* x *SCD*

Asthma is a common comorbidity in sickle cell disease (SCD) and is associated with acute chest syndrome, stroke, pulmonary hypertension, and early mortality.

→ SCA patients presented significantly elevated absolute eosinophil numbers.

→ Eosinophils isolated from these individuals demonstrated a significantly greater adhesion (70% increased) to fibronectin, an association of the VLA-4, LFA-1, and Mac-1 integrins mediate the adhesion of SCA eosinophils to fibronectina.

→ Indicating that inflammation processes may further stimulate eosinophil adhesion in these patients.

# AIM

Patients with sickle cell anemia during hydroxyurea treatment or not :

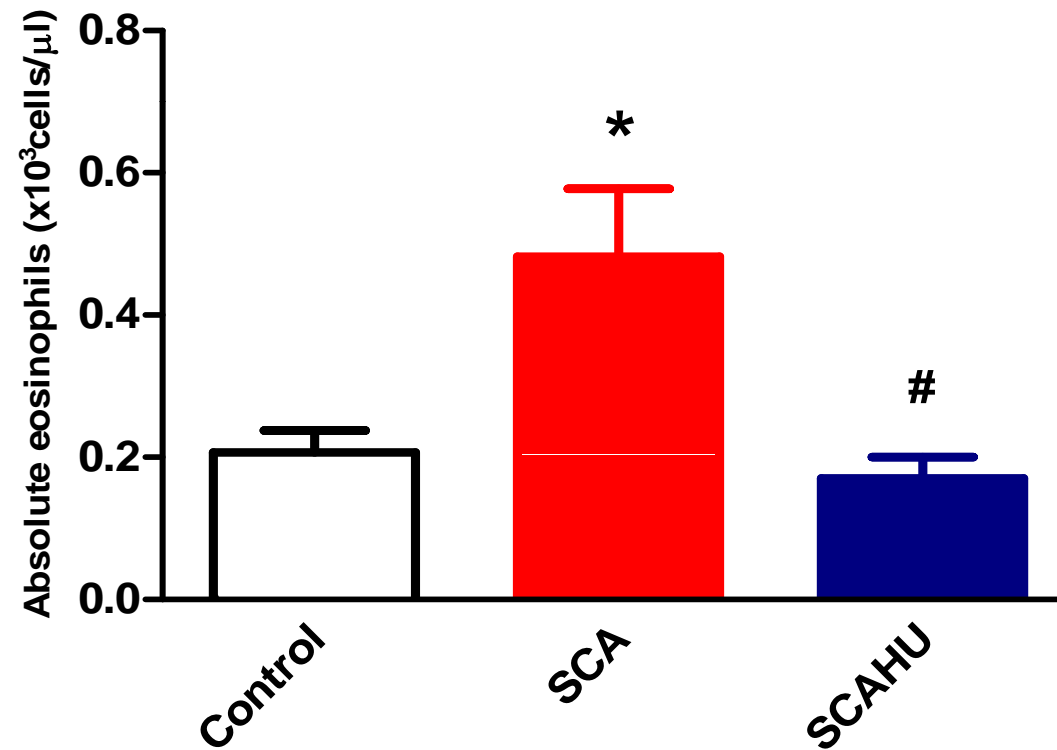
- Evaluate the adhesive properties,
- Chemotactic capacities;
- Degranulation of eosinophils.

# **METHODS**

- Patients and Controls
- Isolation of eosinophils (Percoll gradient and immunomagnetic separation)
- Eosinophil adhesion to fibronectin (Static adhesion)
- Chemotaxis of eosinophils (ChemoTX chamber)
- Degranulation of eosinophils (Eosinophil peroxidase release)

# **RESULTS**

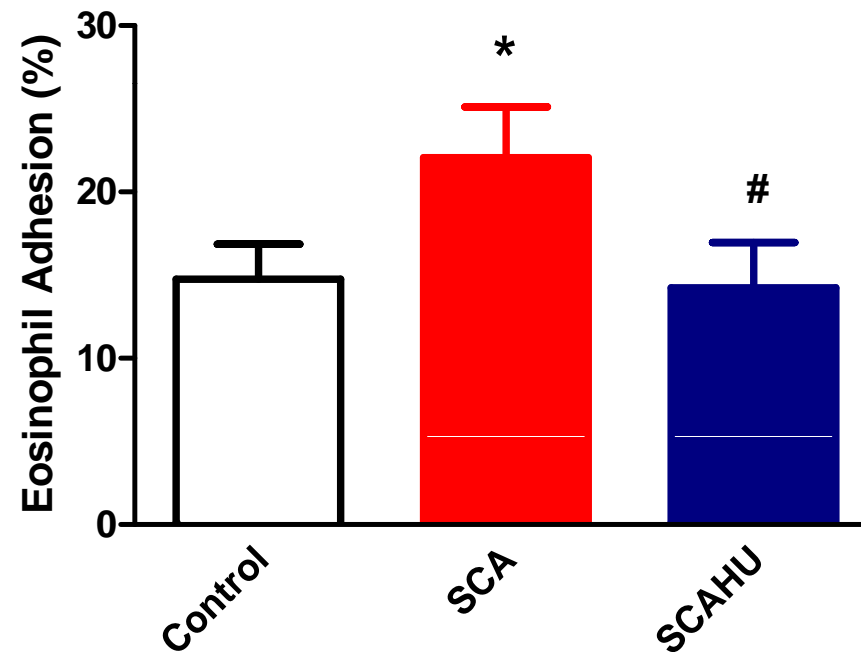
# EOSINOPHIL COUNTS IN WHOLE BLOOD SAMPLES OF SCA PATIENTS



\*P<0.05 compared to control  
#P<0.05 compared to SCA

Control: n<sub>≤</sub>18  
SCA: n<sub>≤</sub>12  
SCAHU: n<sub>≤</sub>13

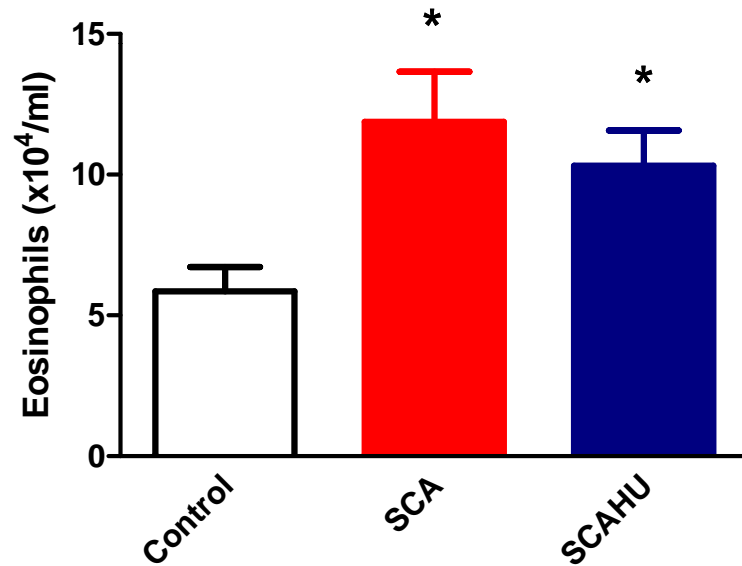
# BASAL EOSINOPHIL ADHESION TO FIBRONECTIN



\*P<0.05 compared to control  
#P<0.05 compared to SCA

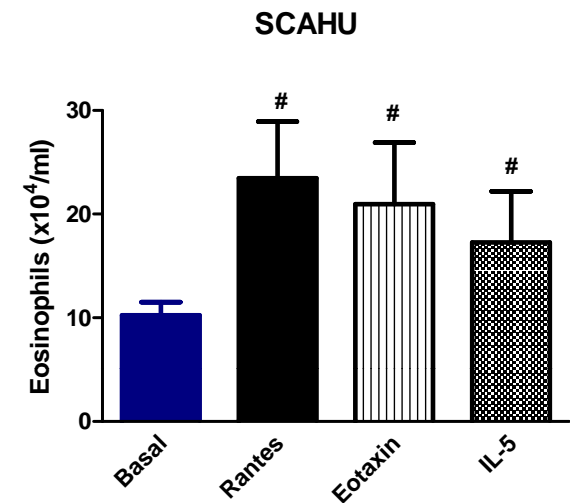
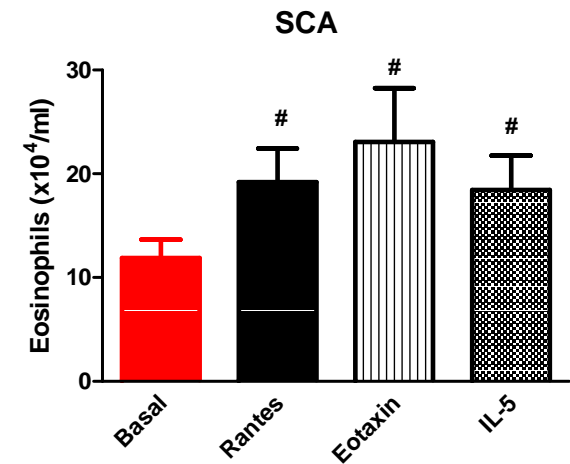
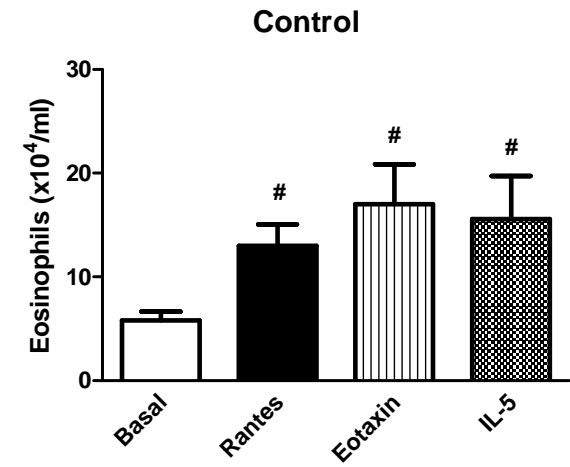
Control: n<sub>≤</sub>18  
SCA: n<sub>≤</sub>12  
SCAHU: n<sub>≤</sub>13

# ***EOSINOPHIL CHEMOTAXIS***

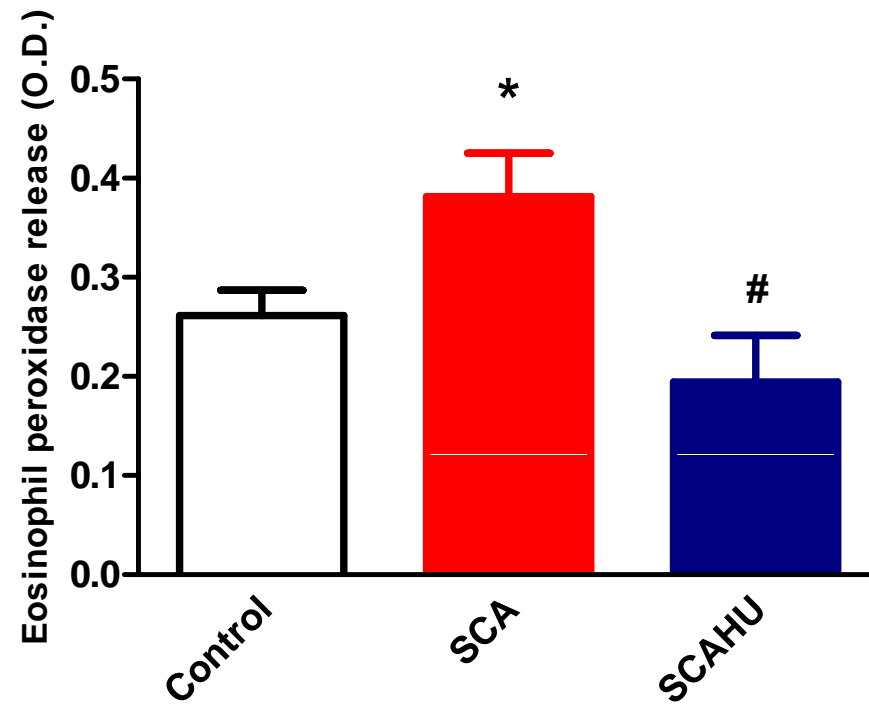


\*P<0.05 compared to control  
#P<0.05 compared to basal

Control: n<sub>≤</sub>18  
SCA: n<sub>≤</sub>12  
SCAHU: n<sub>≤</sub>13



# **EOSINOPHIL DEGRANULATION**



\*P<0.05 compared to control

#P<0.05 compared to SCA

Control: n<sub>≤</sub>18  
SCA: n<sub>≤</sub>12  
SCAHU: n<sub>≤</sub>13

# **CONCLUSIONS**

- These are the first data to show that eosinophils from patients with sickle cell anemia have higher chemotactic and degranulation capacities;
- Therapy with hydroxyurea is associated with reduced adhesion and degranulation of eosinophils of these patients, but has no effect on chemotactic ability of these cells;
- These data suggest that in addition to neutrophils, eosinophils may also play a role in the pathophysiology of the disease.