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## Neutrophil Gelatinase-Associated Lipocalin Increases HLA-G<sup>+</sup>/FoxP3<sup>+</sup> T-Regulatory Cell Population in an In Vitro Model of PBMC

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## Introduction

- NGAL = Neutrophil gelatinase-associated lipocalin = lipocalin 2
- NGAL is a glycoprotein, which belongs to the family of „lipocalins“
- Lipocalins transport small hydrophobic molecules into the cells
- Lcn-2 expression:
  - Neutrophils, Macrophages, Dendritic Cells
  - Trachea, Lung, Sputum, BAL, Stomach, Small intestine, Panceas, Kidney, Protate, Thymus
- Functions of NGAL:
  - Modulation of intracellular iron
  - Bacteriostatic activity
  - Antagonist of oxidative stress
- Very high sensivity but low specificity
- Successful Biomarker for ischemic renal damage.
- Biological role is still unclear.

## Introduction

- HLA-G is a HLA class I molecule
- It is the only HLA-class 1 molecule expressed in the placenta
- Important role in fetal-maternal immune tolerance



## Introduction

- FoxP3+T-regulatory cells
- Essential for the balance between pro and anti-inflammatory responses
- Different mechanisms of immunomodulation known
- Important for tolerance in organ and tissue transplantation

## Introduction

- PBMC = peripheral blood mononuclear cell
- Monocytes, Macrophages, Lymphocytes

## Materials and Methods

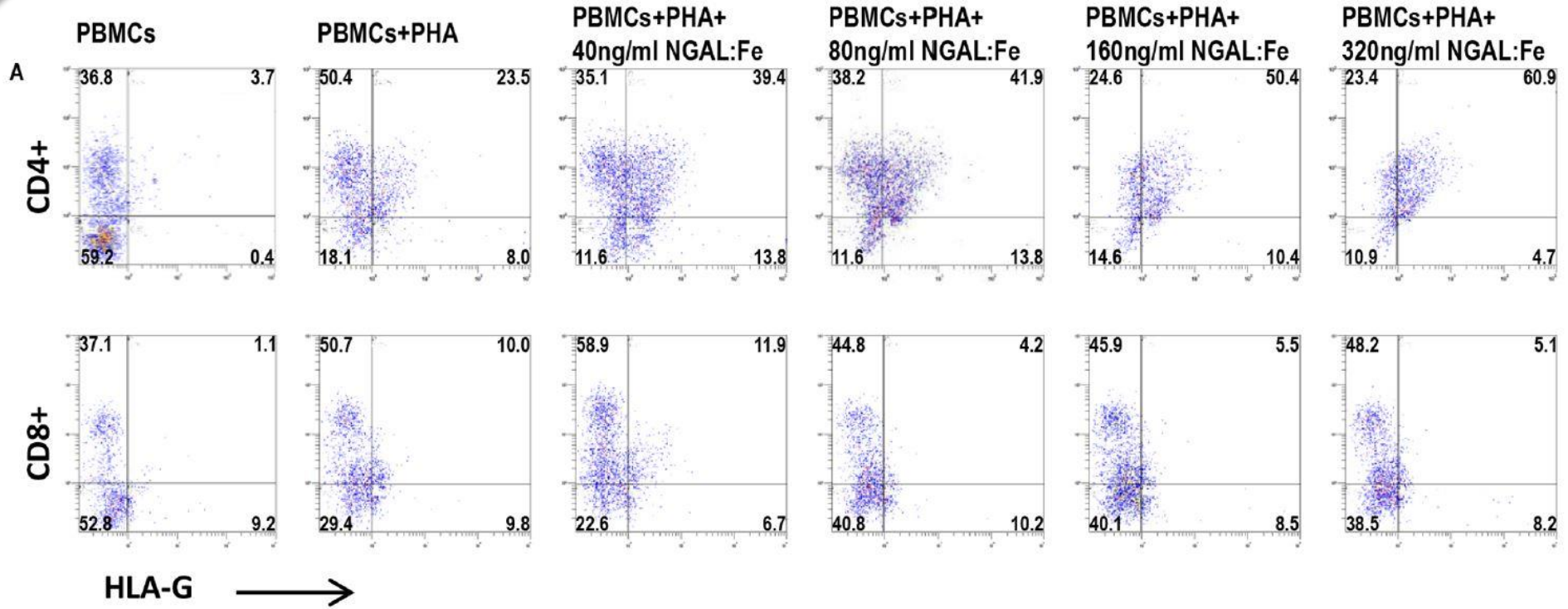
- PBMCs were obtained from 8 healthy donors
- Various NGAL treatments
- Flow cytometry and ELISA analysis of CD4+ and CD8+ cells



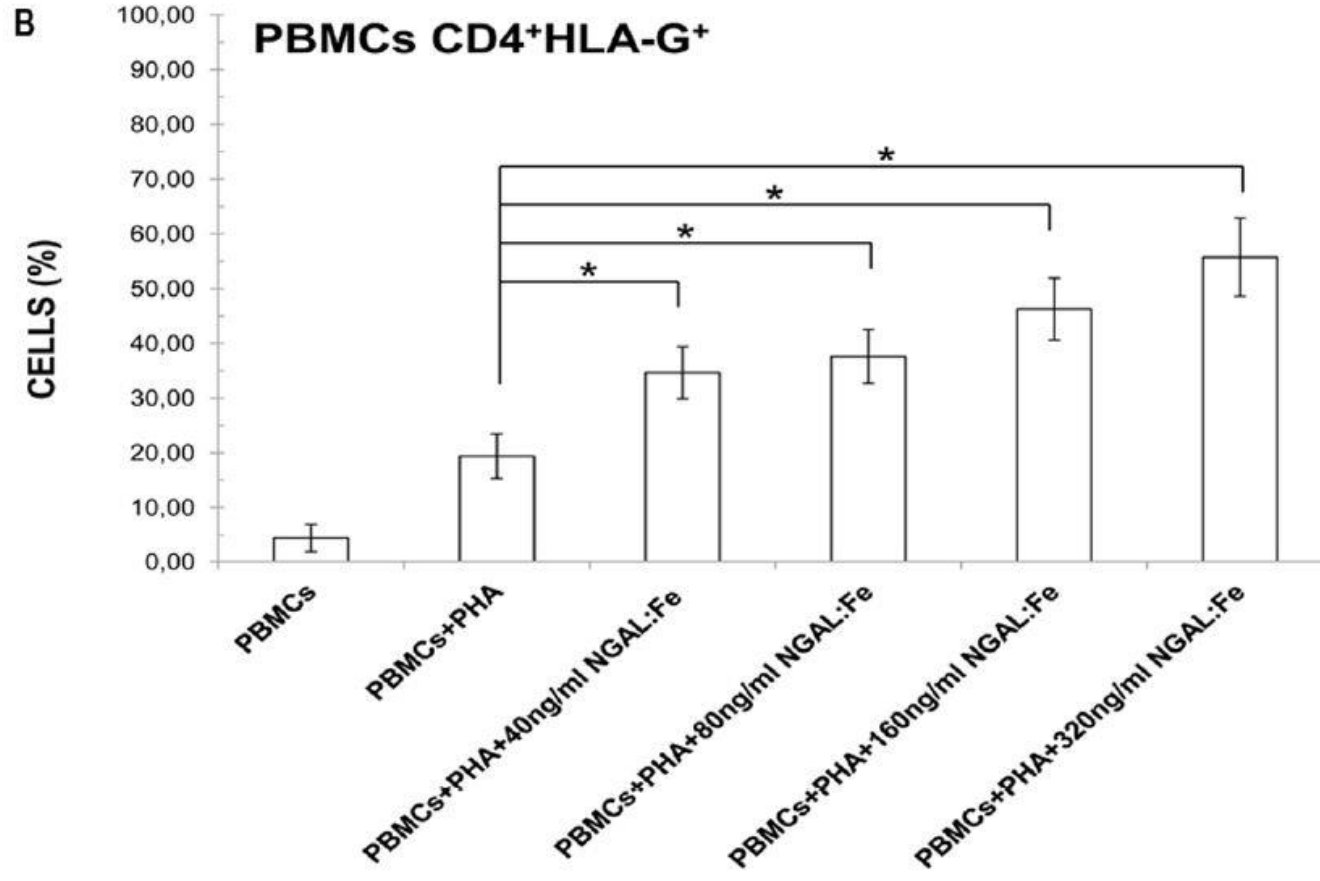
# RESULTS

## 1) HLA-G expression in PHA-activated PBMCs treated with NGAL:enterobactin:iron complex

- PHA = Phytohaemagglutinin = mitogen
- Enterobactin = siderophore, supplies bacteria with iron (NGAL's antioxidant activity may require iron chelation)
- Increased doses of NGAL:enterobactin:iron complex
- Dose dependend increase of HLA-G expression on CD4+ but not CD8+ cells

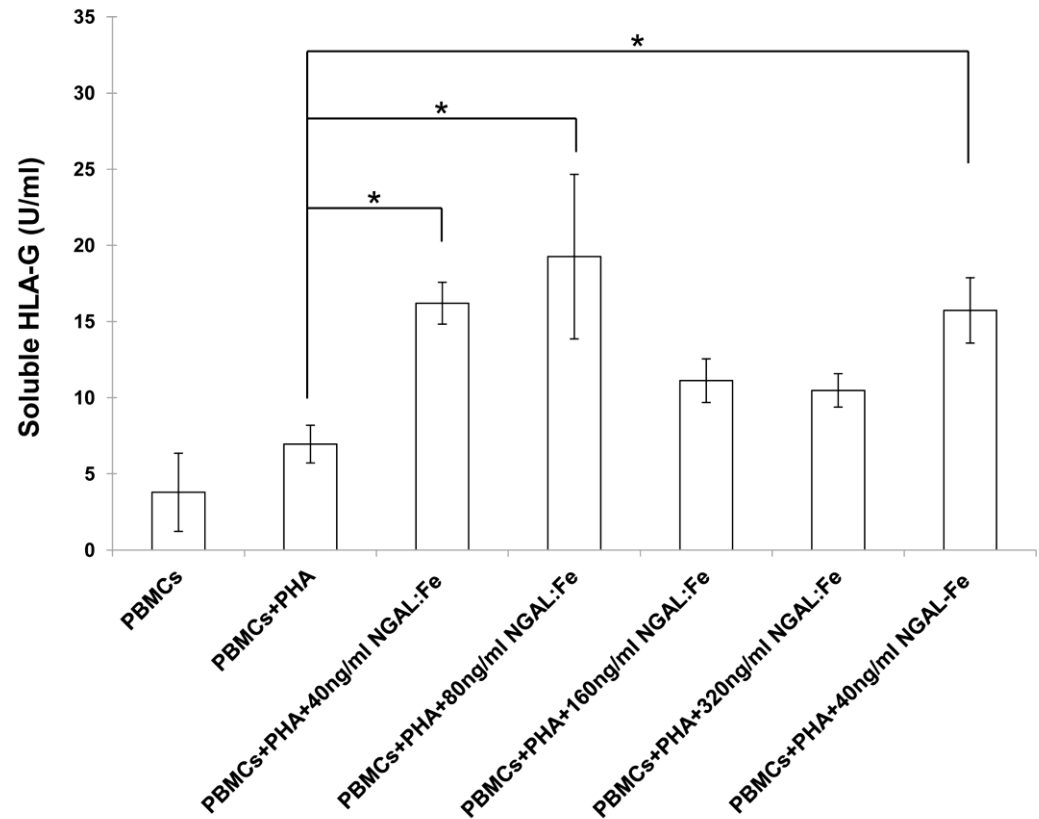






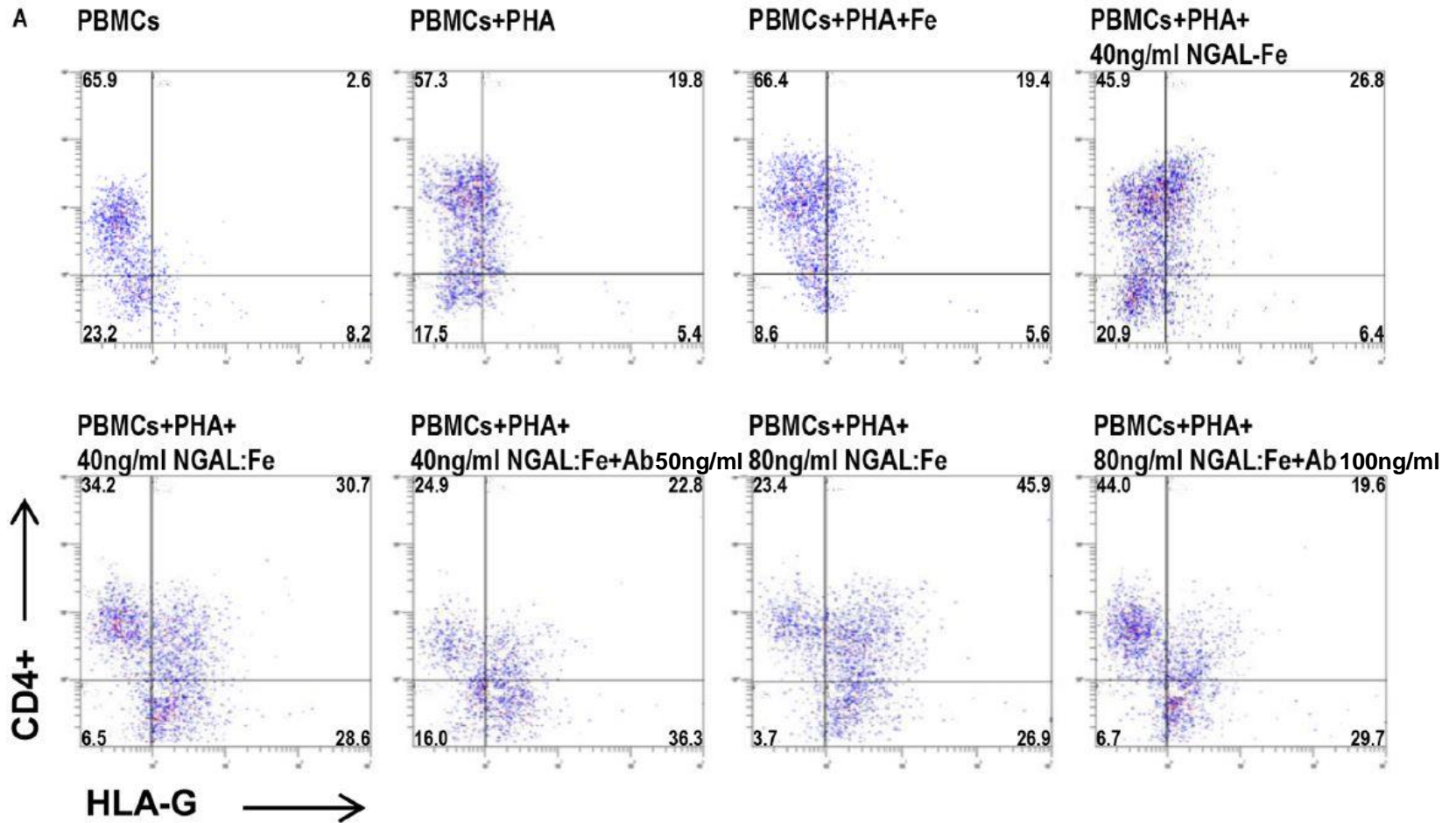
## Soluble HLA-G ELISA

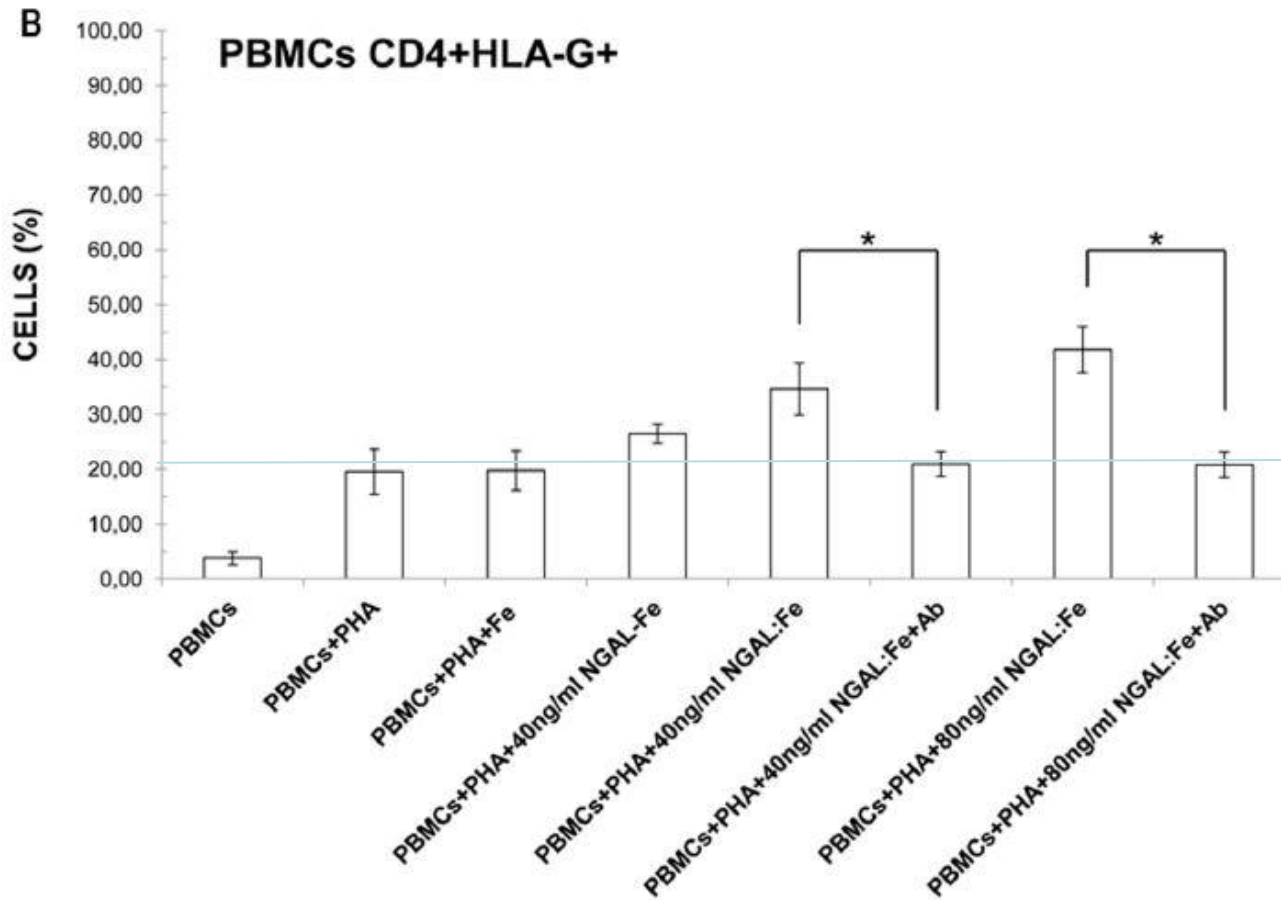
- PBMCs were cultured with 40-320ng/ml NGAL:Enterobactin:Fe complex
- After 72h of incubation supernatants were collected and analyzed



## 2) Evaluation of iron effect and NGAL neutralisation on HLA-G expression in PHA-activated CD4+PBMCs

- Iron without NGAL → didn't increase percentage of HLA-G+ CD4+ cells in comparison to activated CD4+ cells
- NGAL:enterobactin (without iron) → slightly raised expression of HLA-G
- Treatment with NGAL-mAb → significantly decreased percentage of CD4+HLA-G+ cells

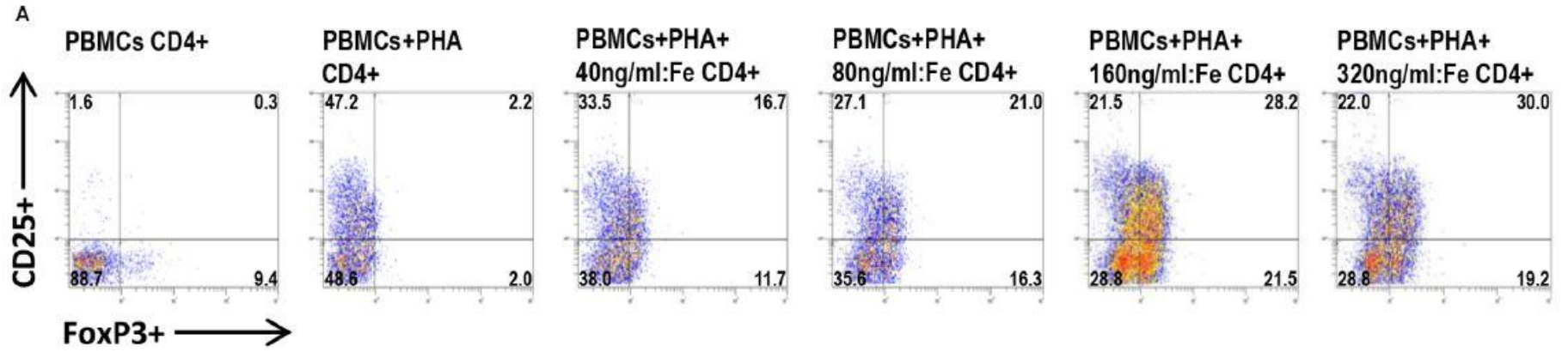


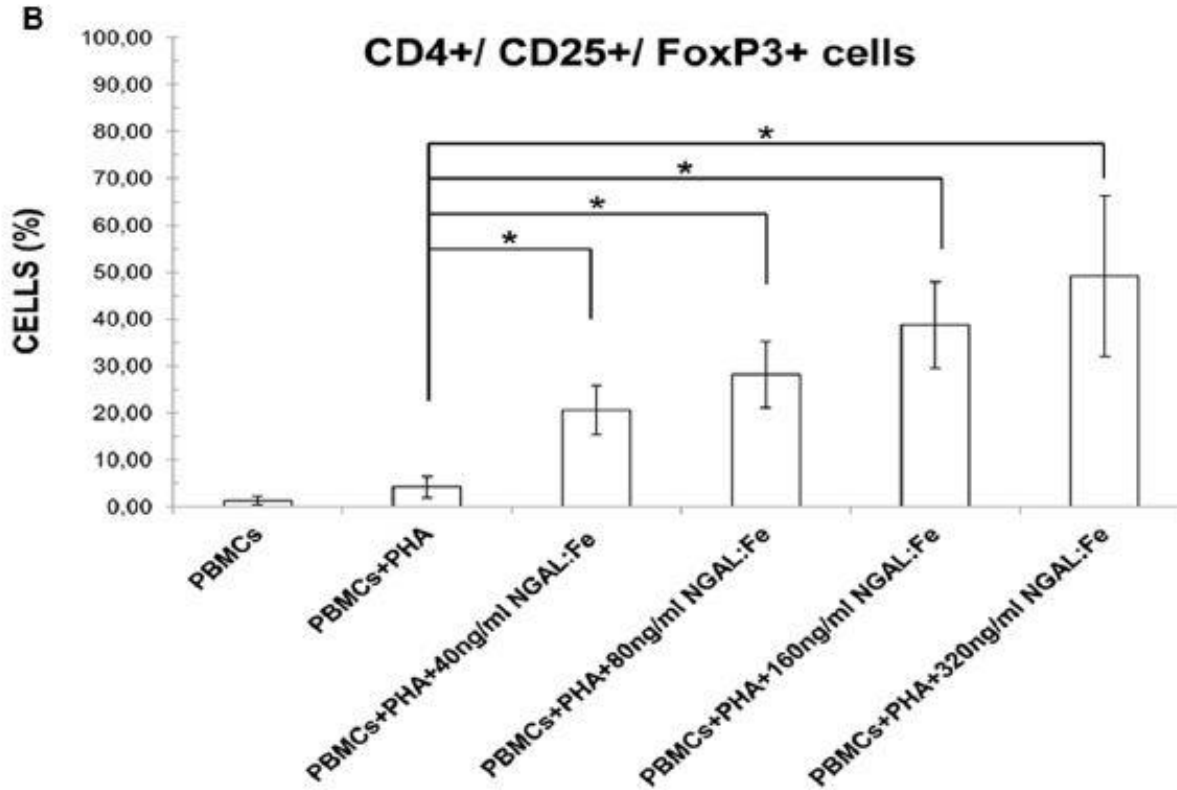




### 3) CD4+/CD25+/FoxP3+ cells in PHA activated PBMCs treated with NGAL:enterobactin:iron complex

- CD4+/CD25+/FoxP3+ cell = T regulatory cell = Treg
- Treatment of PBMCs with increasing doses of NGAL:enterobactin:Iron
- Dose-dependent increase in the percentage of CD4+/CD25+/FoxP3 cells

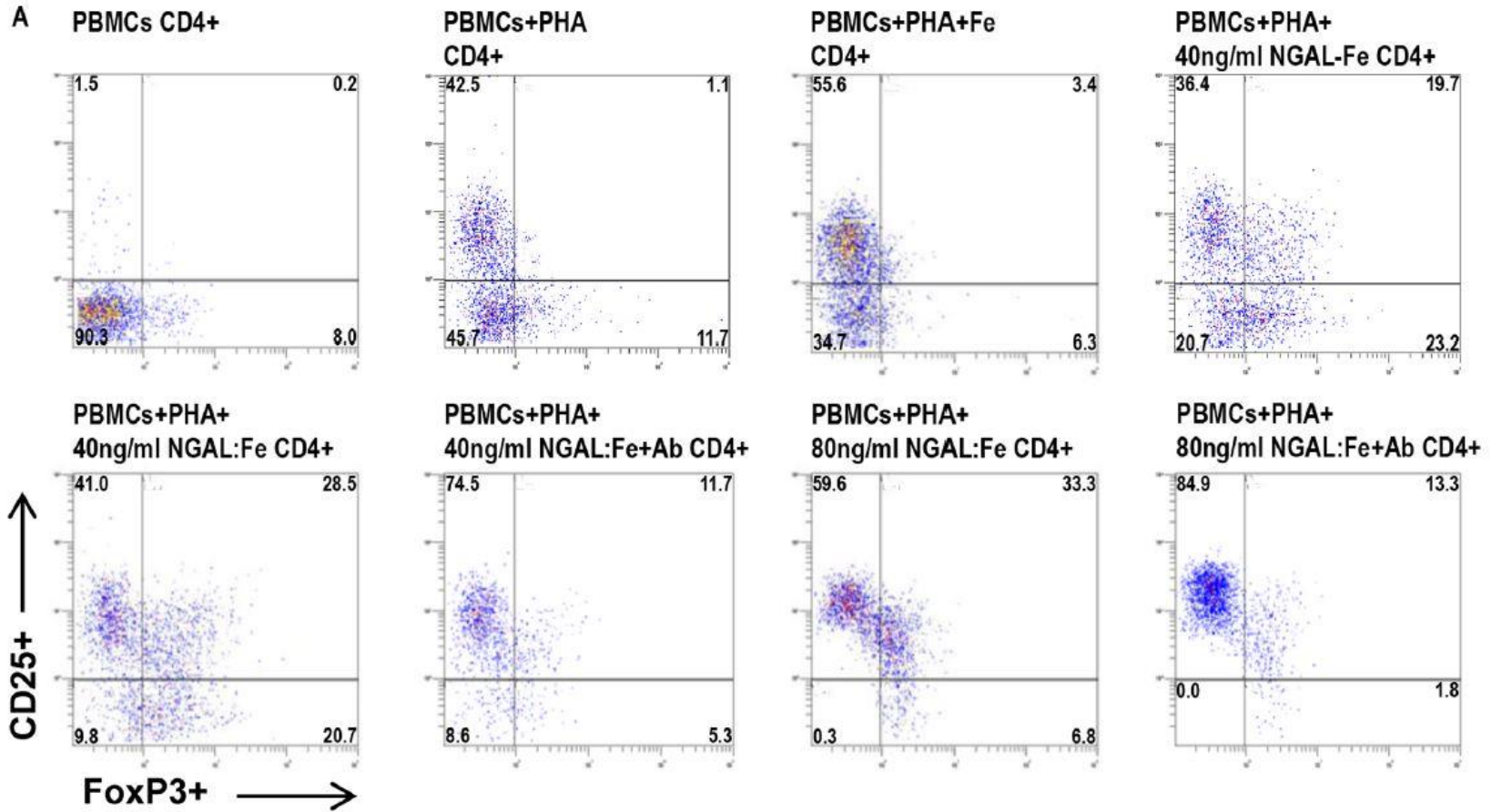


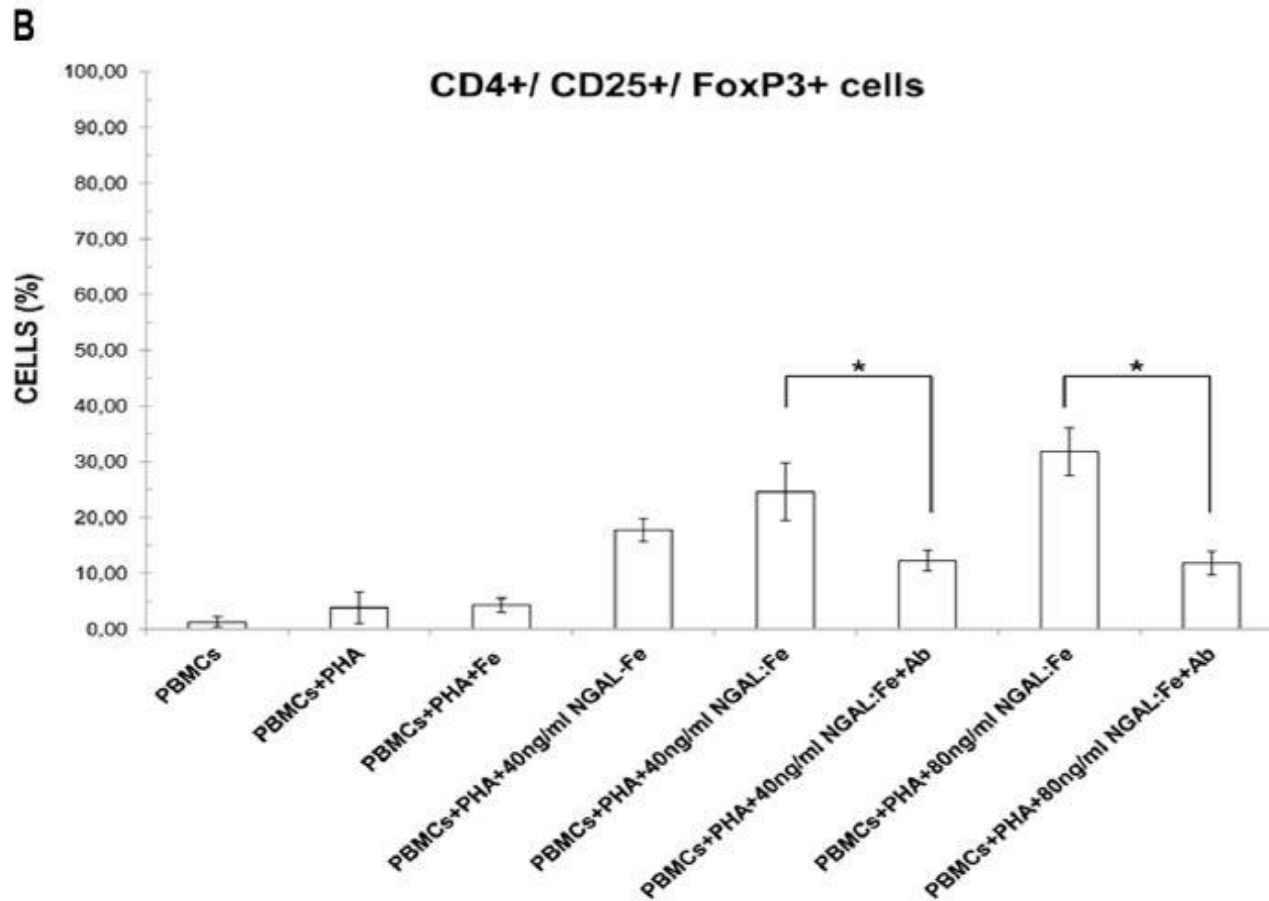




#### 4) Evaluation of iron effect and NGAL neutralization on the CD4+/CD25+/FoxP3+ cell percentage in PHA-activated PBMCs

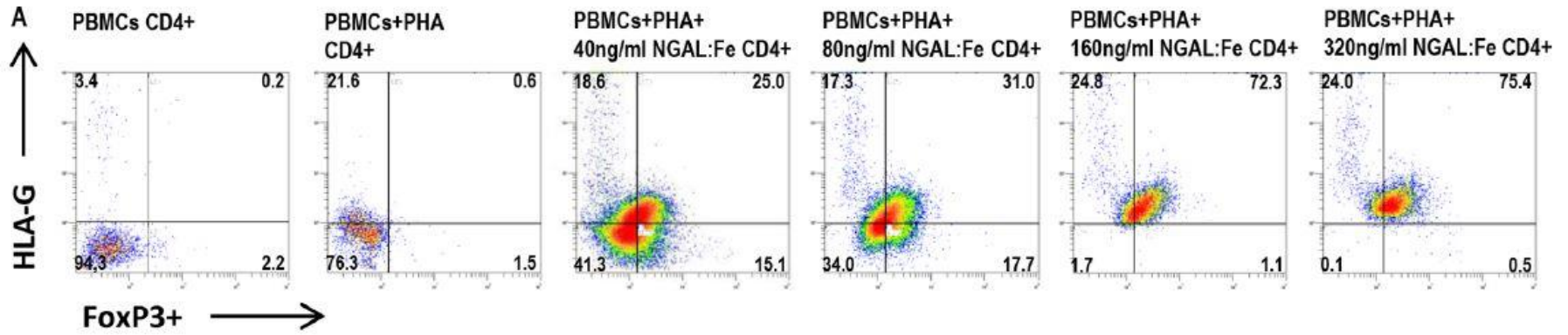
- Iron treatment alone → did not increase percentage of Tregs
- NGAL:enterobactin-Fe → didn't raise expression of Tregs
- NGAL neutralizing Ab significantly reduced expression of Tregs

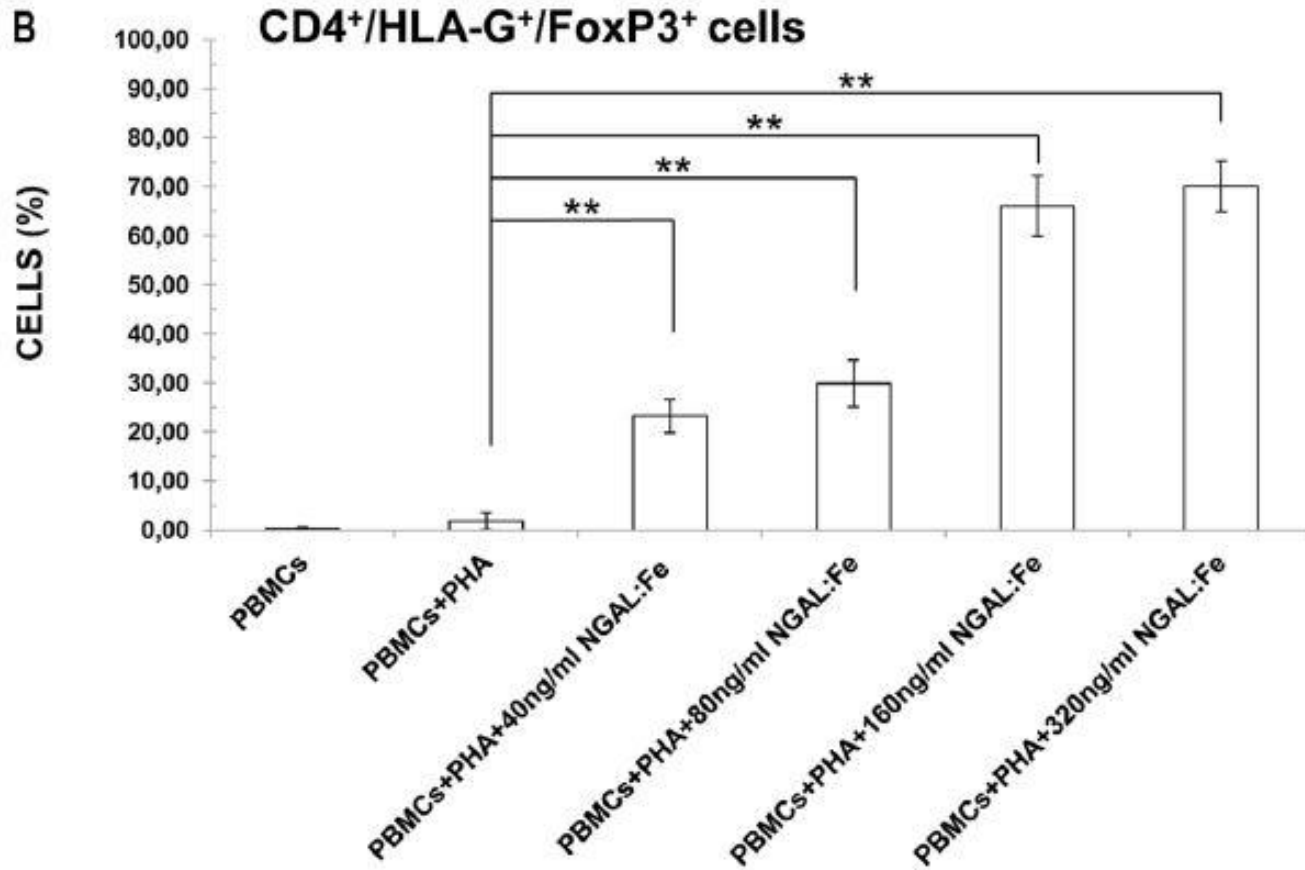




## 5) HLA-G+/FoxP3+ cells in PHA activated PBMCs treated with NGAL:enterobactin:iron complex

- HLA-G+ Tregs can block alloreactivity in vitro, and have been detected after transplantation in vivo.
- They have been shown to have a long-lasting immune-inhibitory effect.
- Added scalar doses of NGAL:enterobactin:iron → dose-dependent increase of HLA-G+/FoxP3+ cells







## SUMMMARY

- Increasing concentrations of NGAL elevate the level of HLA-G expression in CD4+ T lymphocytes
- NGAL doesn't clearly correlate with iron. The absence of iron slightly reduces NGAL action.
- NGAL:Enterobactin:Iron proves unable to modify HLA-G expression in the presence of anti-NGAL antibody
- Increasing doses of iron-loaded NGAL induce dose-dependent expression of CD4+/CD25+/FoxP3+ cells

# DISCUSSION

## *NGAL and Iron*

- NGAL is a major iron-transporting protein, complementary to transferrin
- NGAL provides protective effects in experimental models of acute kidney injury in which reactive oxygen species (ROS) play an important role
- Free iron is one of the most potent factors in ROS generation
- NGAL provides a reservoir for excess iron and may supply a regulated source of intracellular iron to stimulate repair mechanisms
- NGAL reduces iron-mediated toxicity
- Data suggests that NGAL might activate an iron-independent pathway



- NGAL upregulates heme-oxygenase-1 (HO-1)
- Functions of HO-1:
  - reduces iron-uptake
  - promoting intracellular iron release
  - stimulating production of antioxidants
  - inducing expression of cell cycle regulatory protein p21

## *NGAL and NF- $\kappa$ B*

- NGAL is upregulated in an NF- $\kappa$ B related manner by IL-1 $\beta$  stimulation through activation of the MAPK/ERK pathway
- NF- $\kappa$ B plays a key role in innate and adaptive immune systems
- NGAL might have a role in immune tolerance

## CONCLUSION

- NGAL is a mediator of various biological states.
- The biological role of NGAL remains unclear.
- The high sensitivity with low specificity suggests an important biological role.
- NGAL may provide a protective role for several cellular stresses including inflammation and oxidative stress.
- NGAL may play a role in immune tolerance.

**Thank you for your attention!**