Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

Cytokine secretion level of peripheral blood mononuclear cells (PBMCs) after exposure to irradiated PBMC secretome

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Contents

- Background
- Materials and Methods
- Results
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Peripheral Blood Mononuclear Cells (PBMCs)

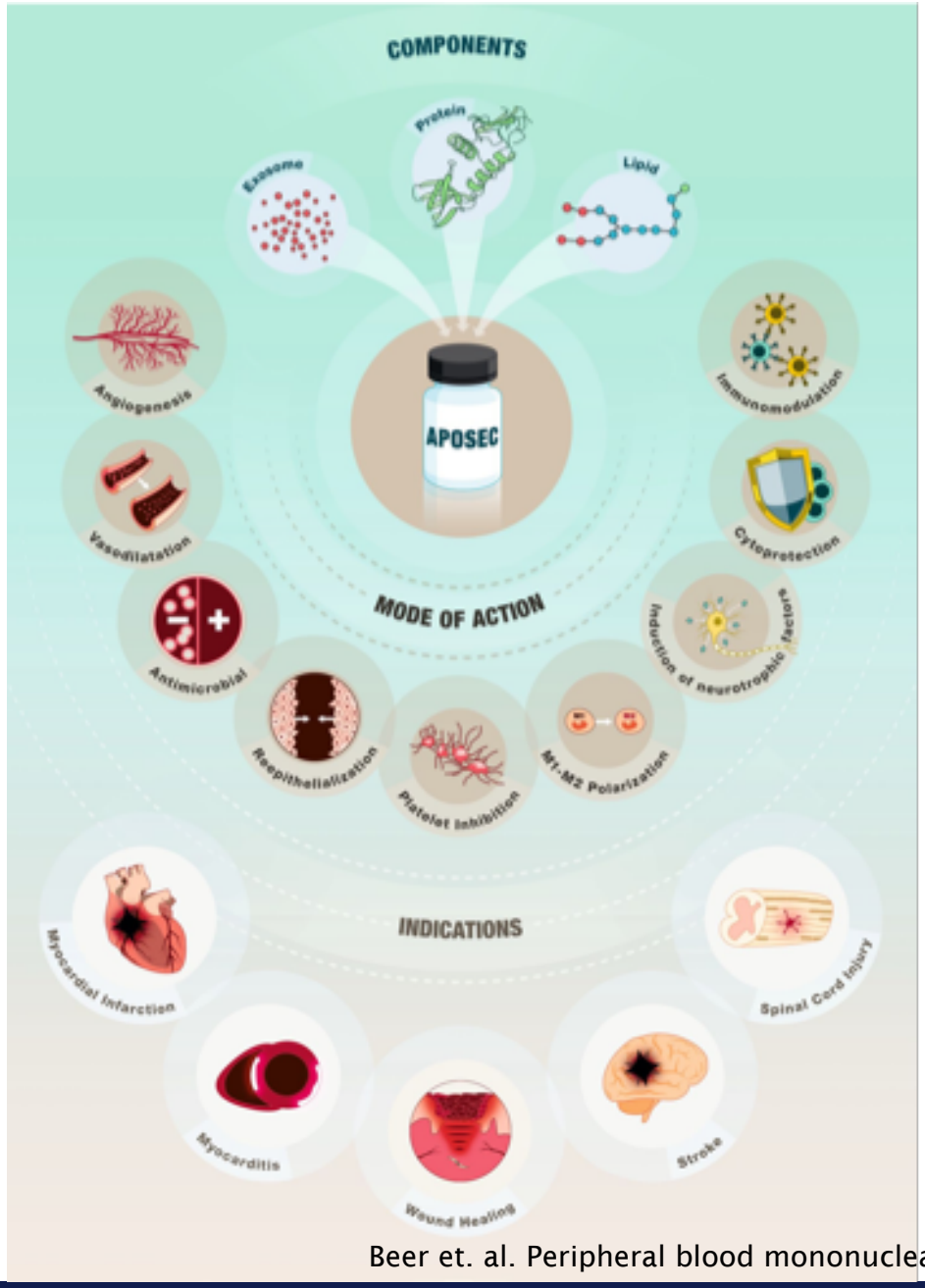
- Include lymphocytes, monocytes, NK - cells and dendritic cells
- Distinction between T – Lymphocytes and B - Lymphocytes
- Cluster of Differentiation

Apoptotic Secretome (APOSEC)

- Gamma – irradiated PBMCs enter programmed cell death

- Immunomodulatory effects are put in motion
  - cytoprotection, release of growth factors, angiogenesis

- Vast array of active agents
  - proteins
  - extracellular vesicles (microparticles, exosomes)
  - lipids



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Preclinical and clinical application of APOSEC

Species	Experimental model	Effects on disease	Application	Concentration at cultivation	PBMC source	Apoptotic stimulus	References
Rat	AMI	Reduced infarct size, improved functional parameters	i.v.	25×10^6	Syngen	γ-irradiation (60 Gy)	[98]
Pig	AMI		i.v.	25×10^6	Syngen	γ-irradiation (60 Gy)	[97]
Pig	AMI		i.v.	25×10^6	Syngen	γ-irradiation (60 Gy)	[99]
Mice	EAM	Resolution of acute inflammation	i.p.	25×10^6	Syngen	γ-irradiation (60 Gy)	[100]
Mice	Dermal wound	Improved wound healing	Topical	25×10^6	Syngen	γ-irradiation (60 Gy)	[101]
Pig	Chronic HF	Improved functional parameters	i.m.	25×10^6	Syngen	γ-irradiation (60 Gy)	[102]
Rat	Stroke	Reduced infarct size, improved neurological parameters	i.v.	25×10^6	Syngen/ human GMP viral cleared	γ-irradiation (60 Gy)	[103]
Rat	SCI	Reduced trauma size, improved neurological parameters	i.p.	25×10^6	Human GMP viral cleared	γ-irradiation (60 Gy)	[104]
Pig	Dermal wound	Improved wound healing	Topical	25×10^6	Human	γ-irradiation (60 Gy)	[105]
Pig	AMI	Reduced infarct size, improved functional parameters	i.v.	25×10^6	Syngen GMP viral cleared	γ-irradiation (60 Gy)	[60]
Human	Dermal wound	Safety and tolerability	Topical	25×10^6	Autologous GMP	γ-irradiation (60 Gy)	ClinicalTrials.gov Identifier: NCT02284360[a]

AMI acute myocardial infarction, *EAM* experimental autoimmune myocarditis, *HF* heart failure, *SCI* spinal cord injury, *i.v.* intravenous, *i.p.* intraperitoneal, *i.m.* intramuscular

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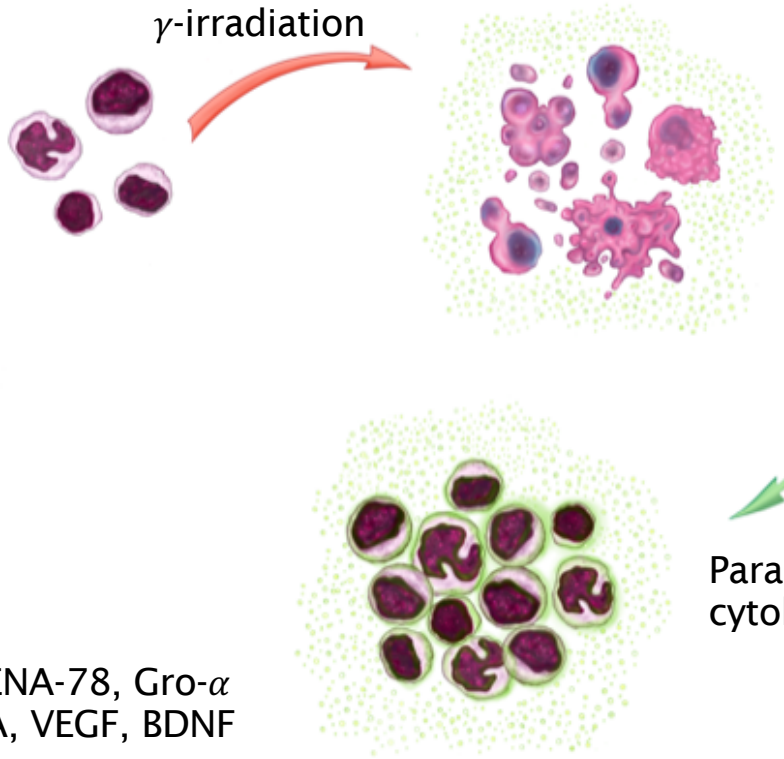
Aims of the study

- Determine whether incubation of viable PBMCs with secretome of irradiated PBMCs alters their cytokine secretion
  → IL-8, ENA-78, Gro-α, VEGF, BDNF, IL-1 RA

- Hypoxic preconditioning and its influence on PBMCs when incubated with secretome of irradiated PBMCs



designed by Daniel Bormann

Materials and Methods

- PBMCs seperation and stimulation

- Hypoxic preconditioning of PBMCs

- ELISA for cytokine measurement
  → IL-8, VEGF, Gro-alpha, ENA-78, BDNF, IL-1RA

PBMCs preparation

- Blood samples acquired at the Department of Transfusion Medicine (General Hospital of Vienna)

- Standard protocol for PBMC seperation employed

  →Ficoll gradient centrifugation
  → dilution to a working concentration of 2,5 x10^6 cells/mL
  (CellGRO Cell culture medium)

- Treatment with APOSEC/CellGRO
  (lyophilized GMP-APOSEC/CellGRO, diluted with NaCl)

- untreated PBMCs served as control

- Incubation in cell culture/hypoxic chamber

Cell cultivation

Normoxic conditions

- Seperation and treatment of cells → transfer to cell culture (24h)

- Time dependancy

  set checkpoints for cell removal (6h/12h/18h/24h)

Hypoxic conditions (1% O_2)

- Seperation and treatment of cells → hypoxic chamber (1h) → transfer to cell culture

- Seperation of cells → hypoxic chamber (1h) → treatment with tested compounds → transfer to cell culture (23h)

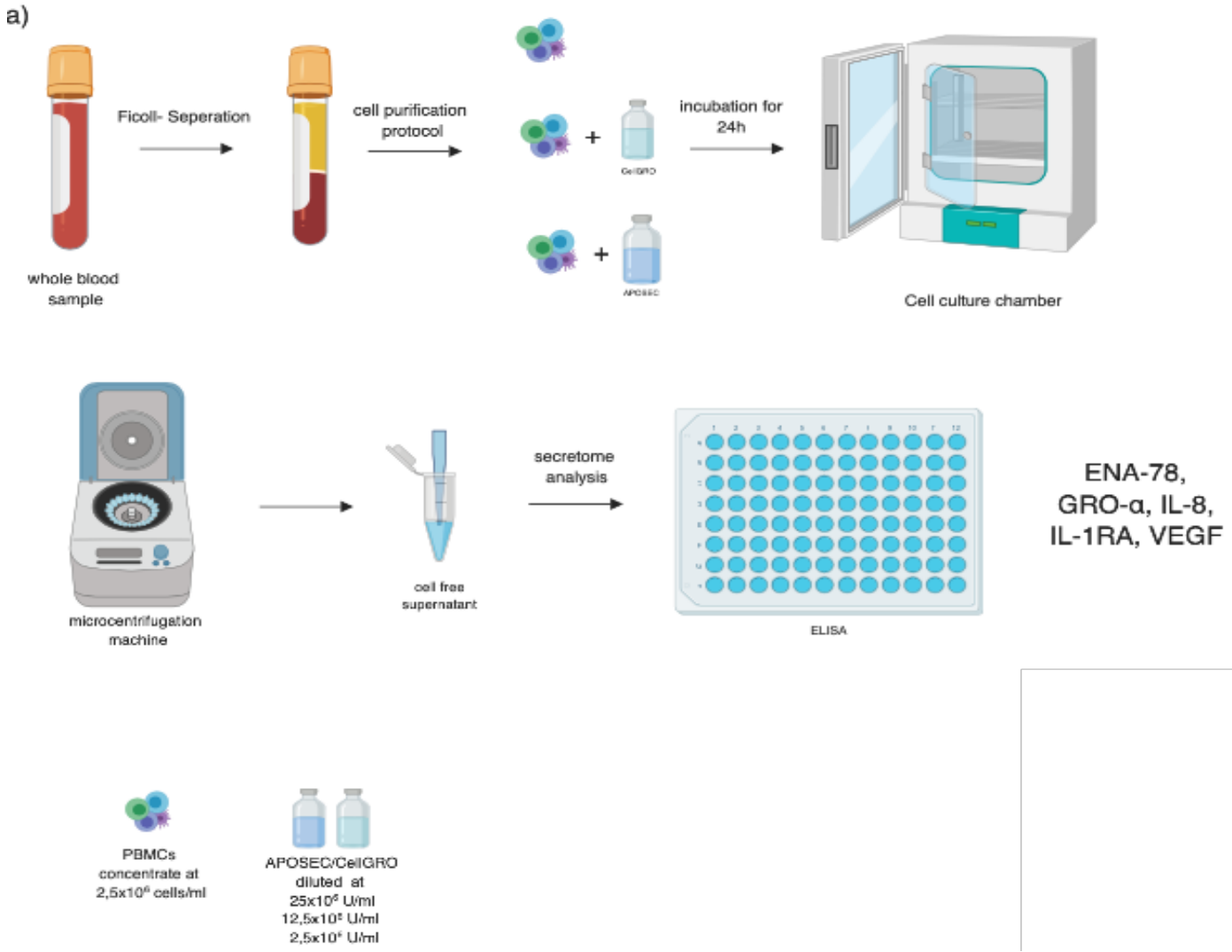
Superantant skimming

- All samples were then skimmed and centrifugated at 2500 rpm for 2 minutes

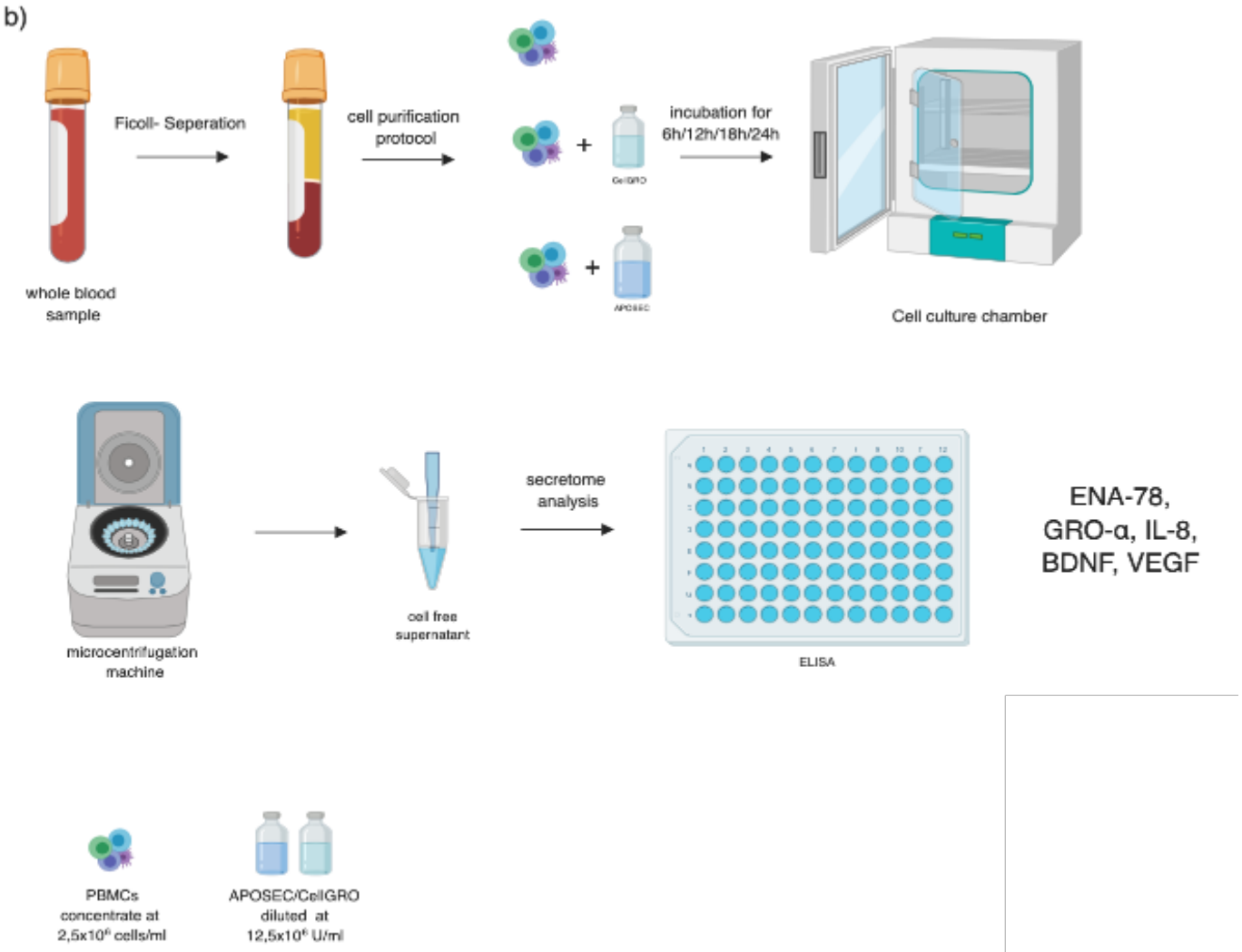
- The supernatants were then separated from the pellets and transferred to separate tubes
  → ELISA for cytokine quantification
  (R&D systems, Duoset kits)

- The pellets were conserved using 500yl of Trizol (TriFAST) to avoid DNA degradation → for future analysis of gene alterations

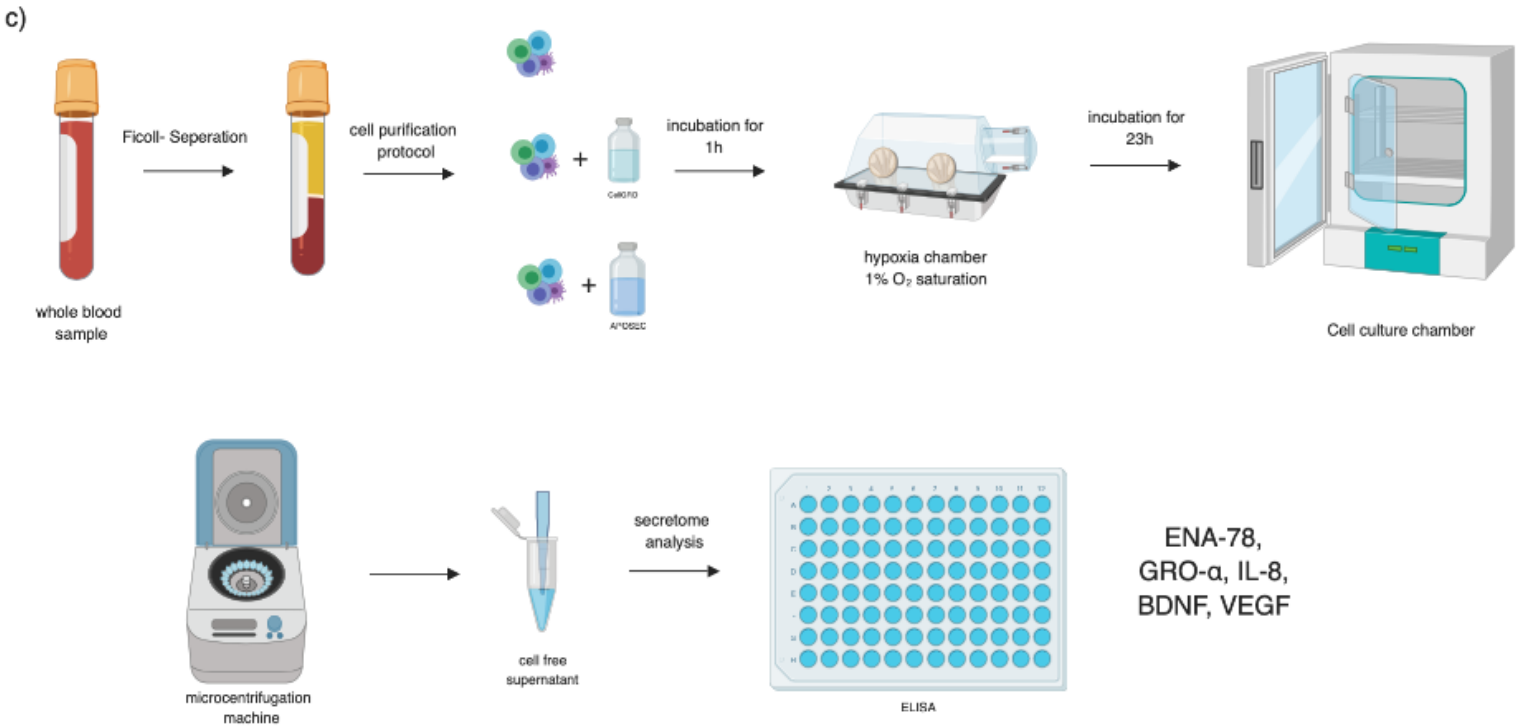
Workflow – normoxic cell culture



Workflow – normoxic cell culture and time-dependancy



Workflow – involving hypoxic chamber



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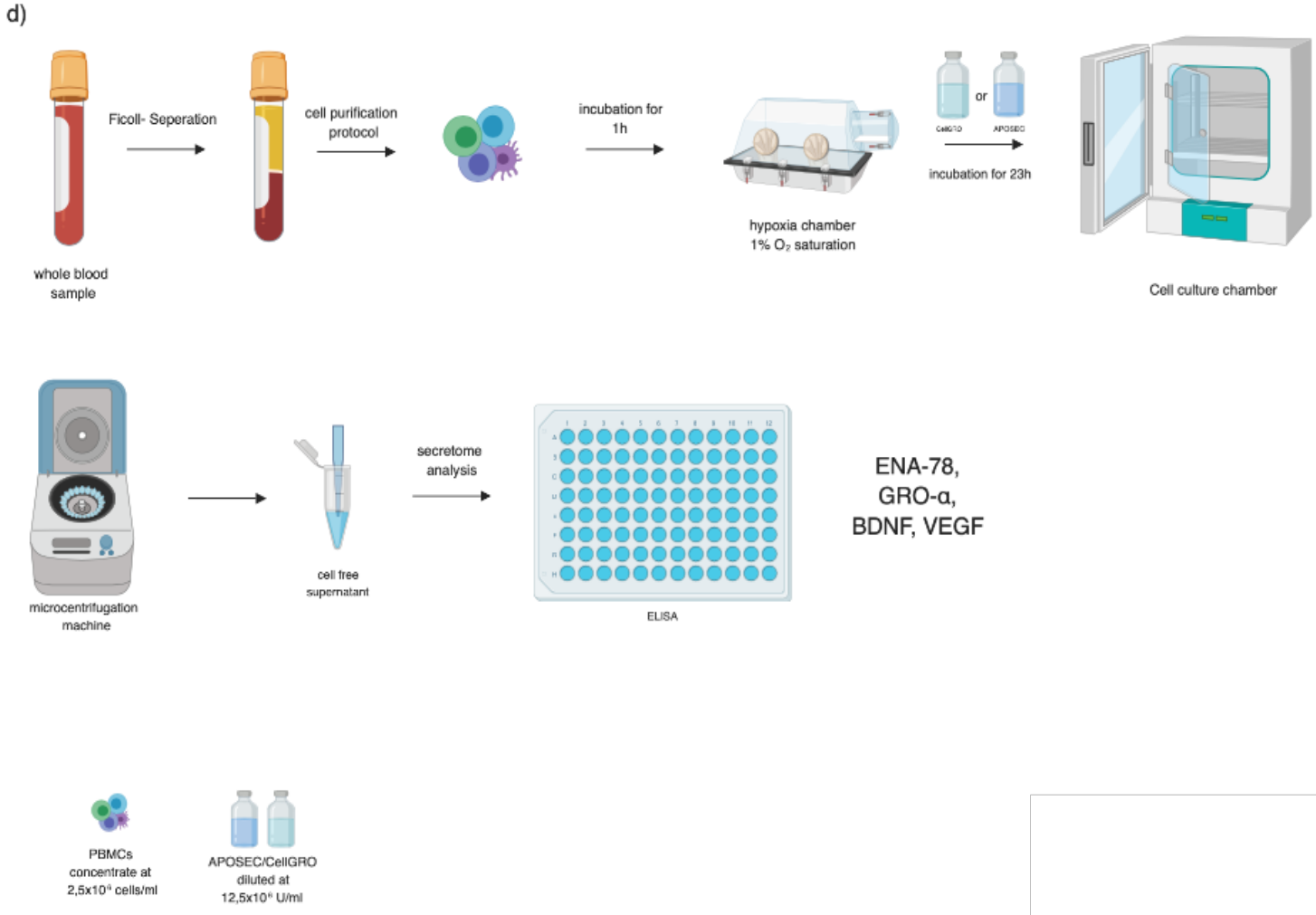


Statistical analysis

- Graph pad prism 5 for evaluation
  - box plots
  - comparing column bar graphs

- Mann – Whitney test (Two – way Anova) for concentration dependancy nonparametric test for comparision of at least 3 groups
- One-way anova for all other settings

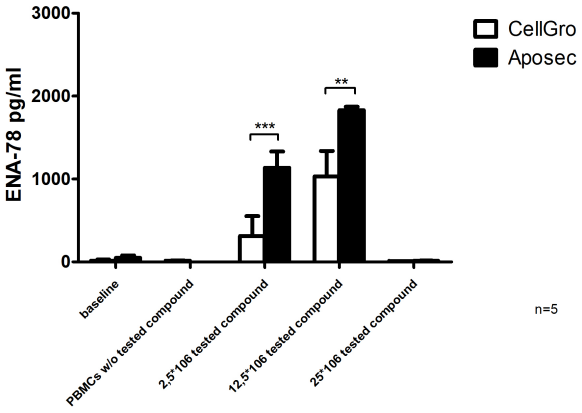
- Dunns multiple comparison test (post-hoc test)
  to pinpoint which specific groups are significant from the other

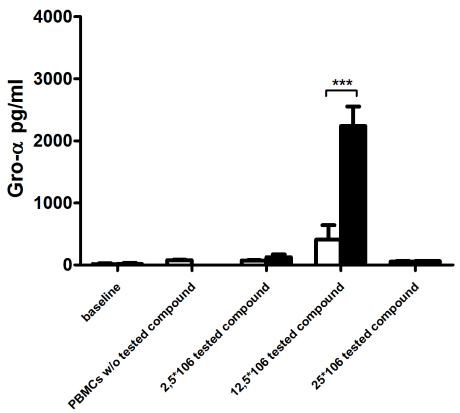
  P< 0,05 → statistically significant (*)
  P< 0,01→ statistically highly significant (**)
  P<0,001 → statistically extremely significant (***)

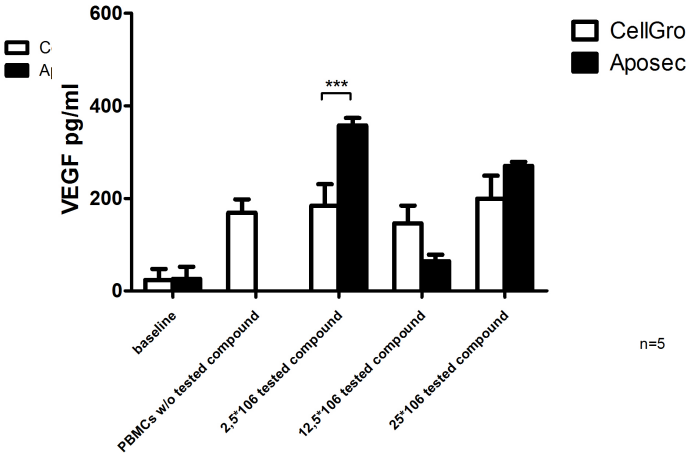
Results

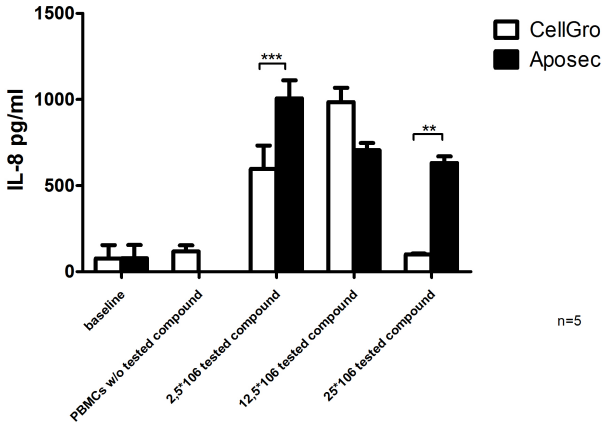
- Cytokine secretion varies with concentration of applied APOSEC/CellGRO
- Cytokine secretion occurs in a time-dependent manner
- Incubation of PBMCs with APOSEC/CellGRO under hypoxic conditions alters cytokine secretion
- Addition of APOSEC/CellGRO to hypoxically preconditioned PBMCs results in higher secretion levels compared to untreated control

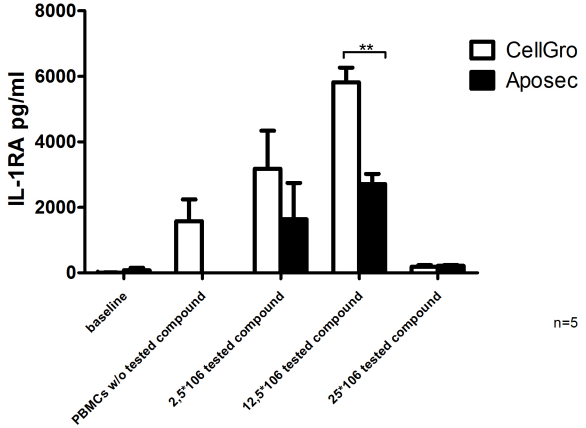
Cytokine secretion varies with concentration of applied APOSEC/CellGRO



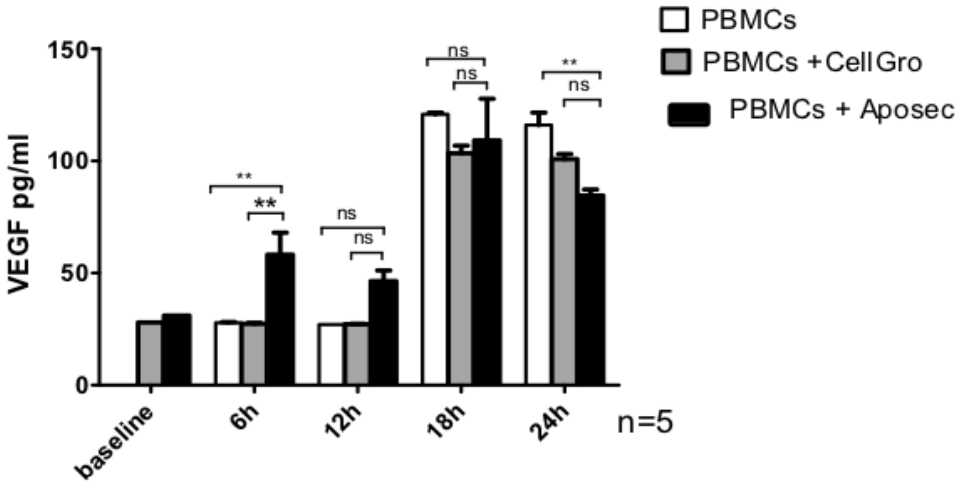


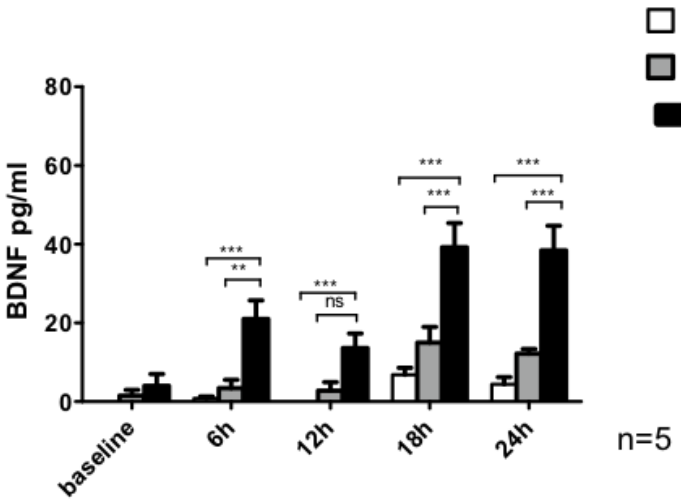


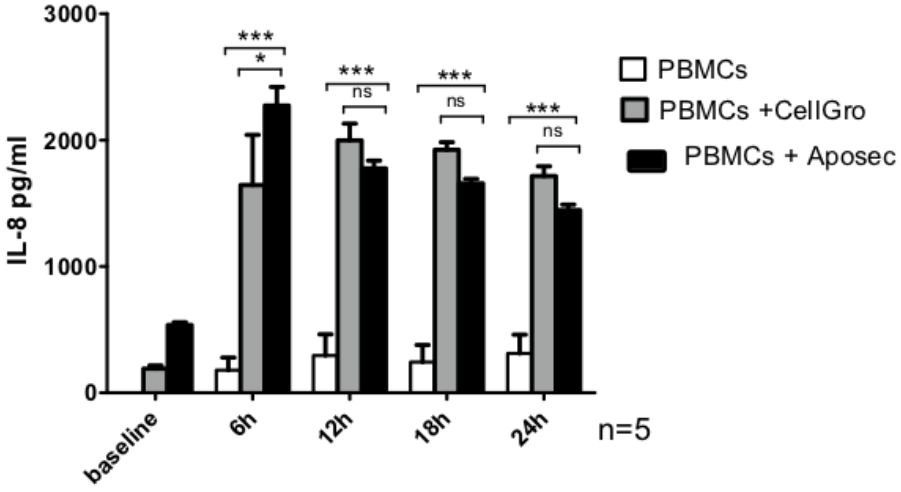




Cytokine secretion occurs in a time-dependent manner







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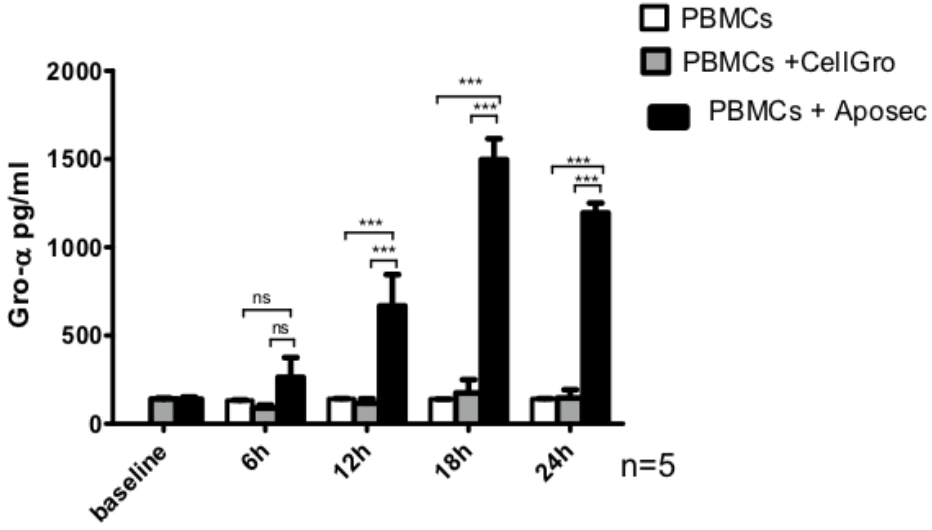
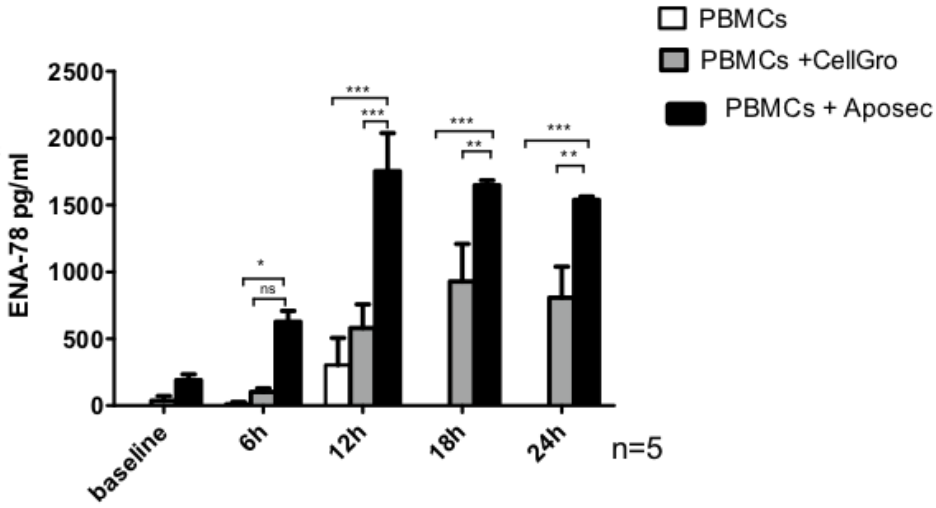
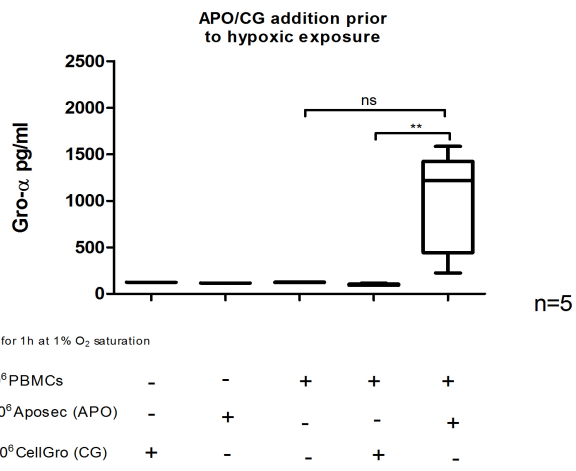
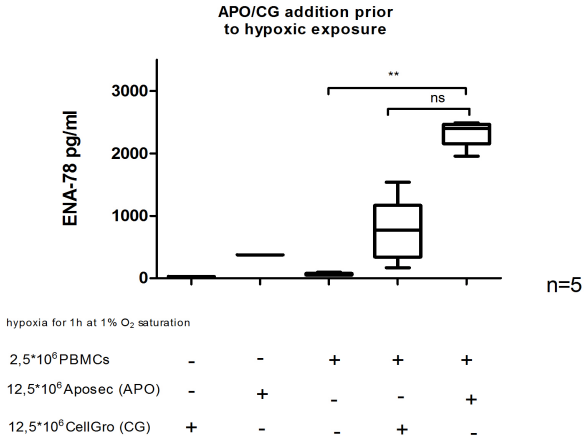
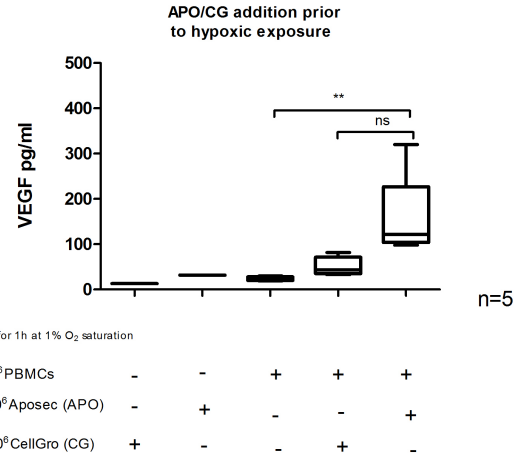


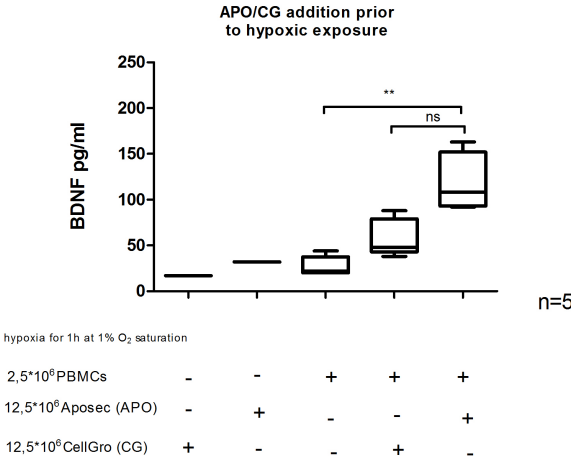


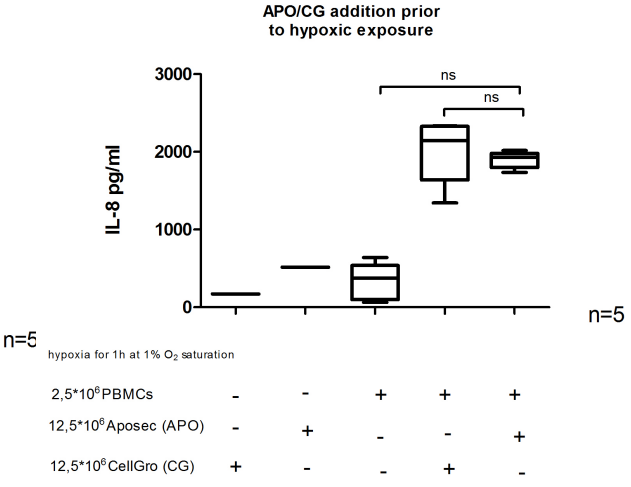
Incubation of PBMCs with APOSEC/CellGro under hypoxic conditions alters cytokine secretion



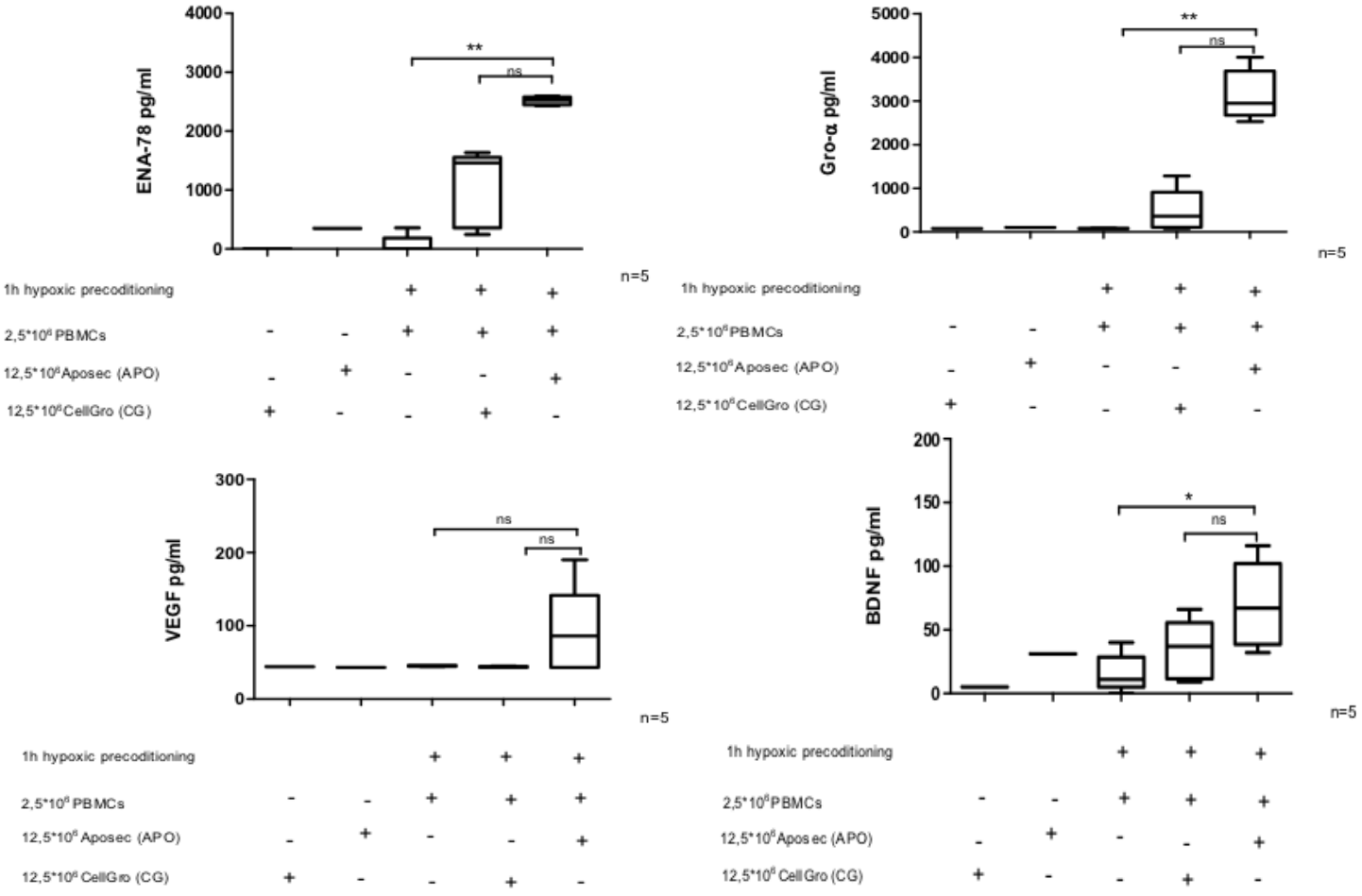








Hypoxic preconditioning increases levels of cytokine secretion when APOSEC/CellGRO is added subsequently



Conclusion

- Addition of apoptotic PBMCs secretome to viable PBMCs resulted in increased cytokine secretion
  → possibly enhancing cytoprotection, angiogenesis

- Samples treated with APOSEC showed higher secretory capacity compared to untreated samples when challenged with hypoxia

- Gene analysis required to provide evidence of active protein biosynthesis

Thank you for your attention