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INTERNATIONAL CLINICAL RESEARCH CENTER

The immune system as source of diagnostics marker and therapeutic targets

IMMUNOPHENOTYPES ASSOCIATED WITH SHORT-TERM SURVIVAL IN PATIENTS WITH SEPTIC SHOCK AND LONG-TERM CHANGES IN IMMUNE SIGNATURE

Jan Frič
Principal Investigator

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Ondřej Mrkva
Jana Bartoňová

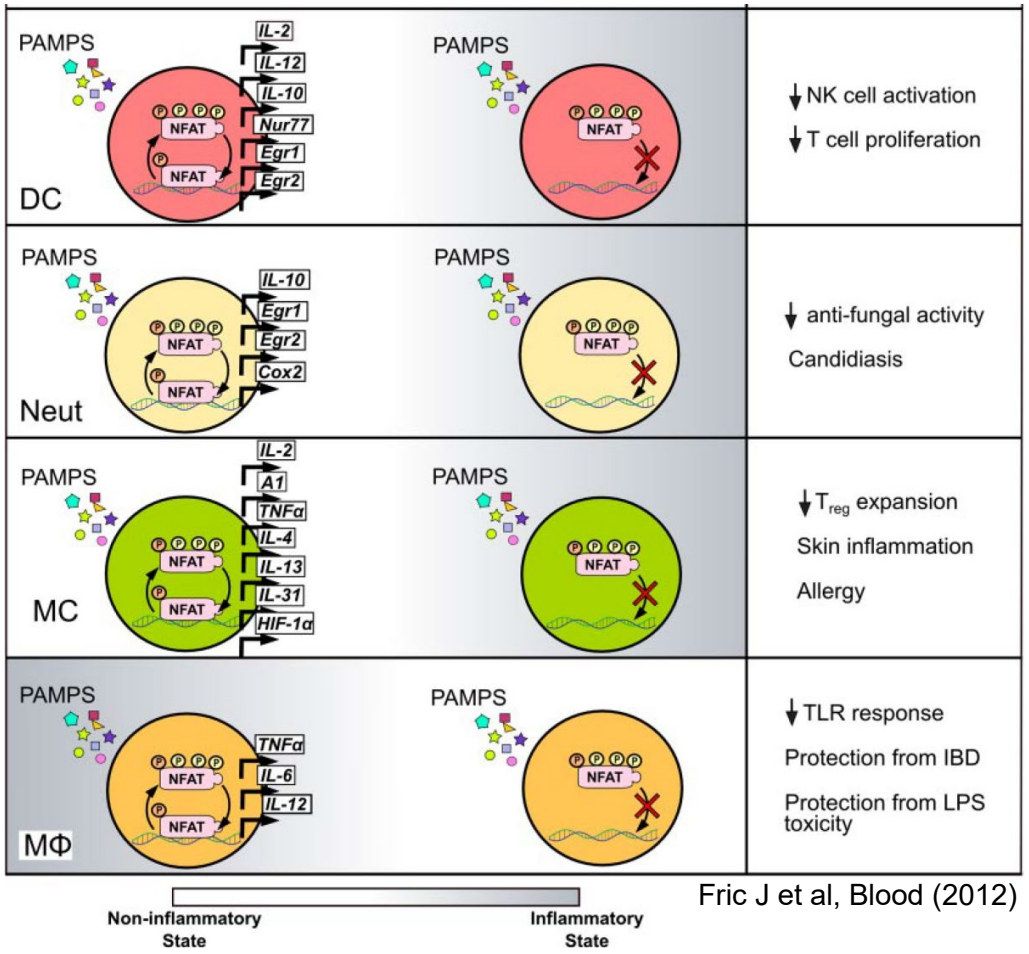
CENTER FOR TRANSLATIONAL MEDICINE

Outline

- the importance of the immune system in sepsis
- why sepsis research @FricLab
- diagnostics markers from the immune system
- therapeutic targets originated in the immune system
- Adverse effects of sepsis - trained immunity

BACKGROUND

Myeloid cell signalling during immunosupression



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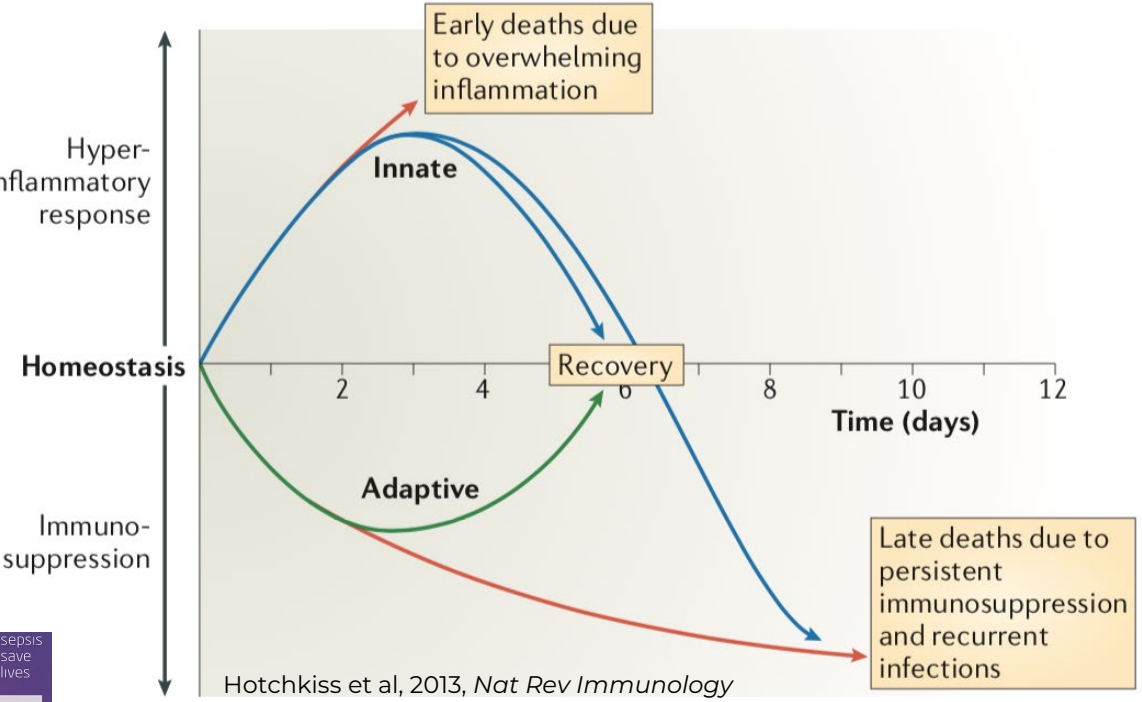
Fric J et al, Blood (2012) 120 (7), 1380-89

Susceptibility of immuno-suppressed patients to infections is due to inhibition of NFAT in myeloid cells (not only T cell).

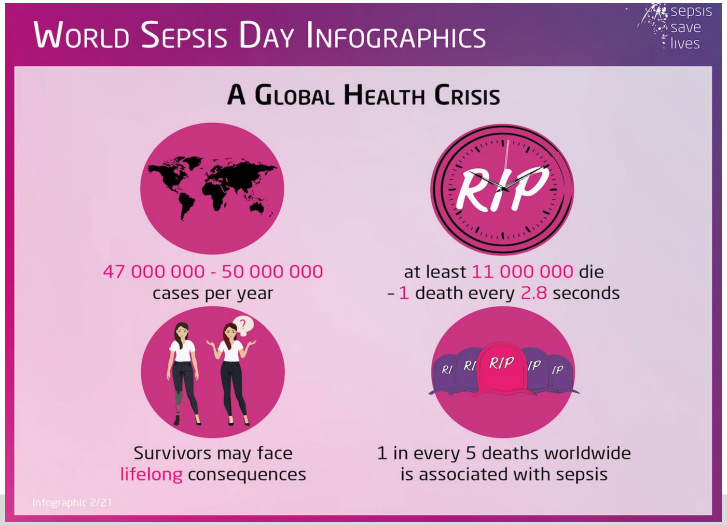
BACKGROUND
Sepsis

Sepsis:

- Life-threatening organ dysfunction;
- Highly **heterogeneous** syndrome;
- Dysregulated **host response** to infection;
- Sustained excessive **inflammation**;
- Immune **suppression;**
- Inflammatory storm followed by immunosuppressive phase
- Pathogens – respiratory (35-68%): Enterococus, Pseudomonas, Candida, Stenotrophomonas
- **No predictable markers:**
- **CRP**
  presepsin,
  procalcitonin
  >200)



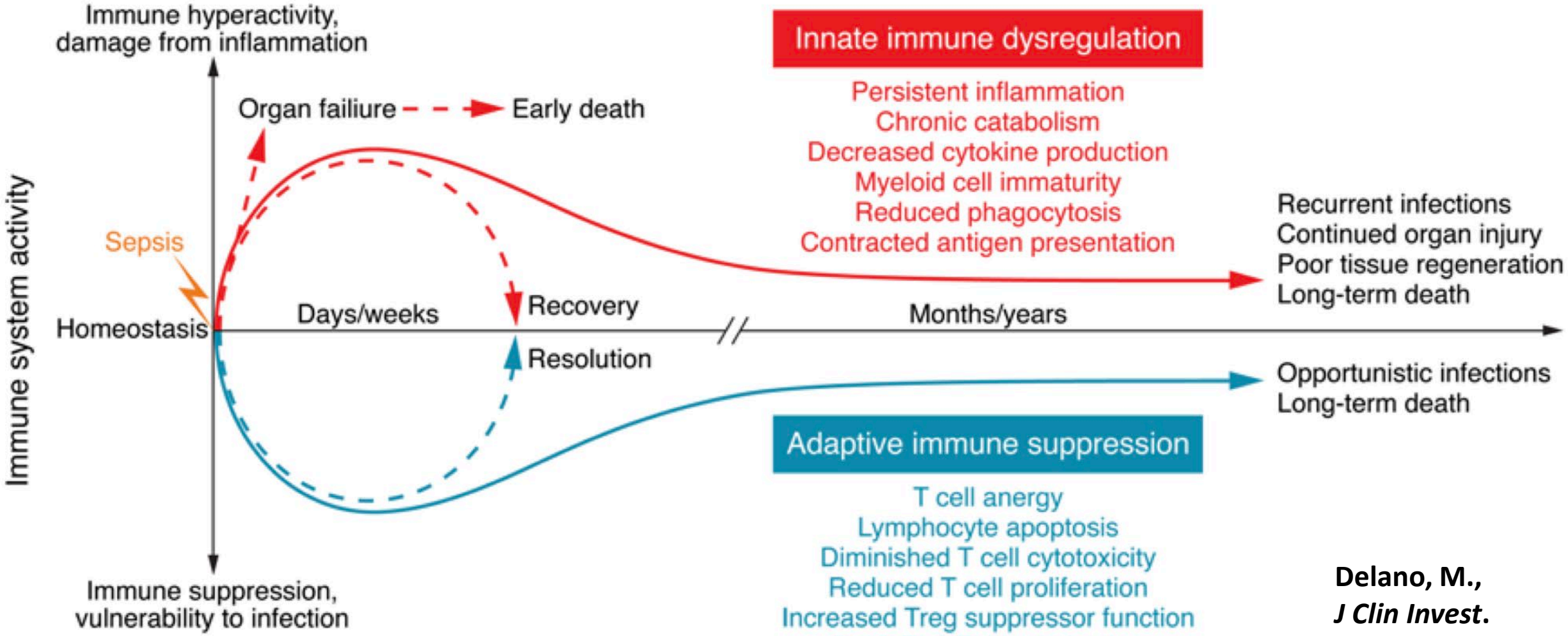
Hotchkiss et al, 2013, *Nat Rev Immunology*



https://www.worldsepsisday.org/sepsis

Source: Rudd et al, 2020 *The Lancet*

BACKGROUND
Immune dysregulation in sepsis



Delano, M.,
***J Clin Invest*.**
2016;126(1):23–31.

BACKGROUND

Sepsis – all immune cells & all cytokines are involved

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DOI: 10.1002/bies.202000067

BioEssays WILEY

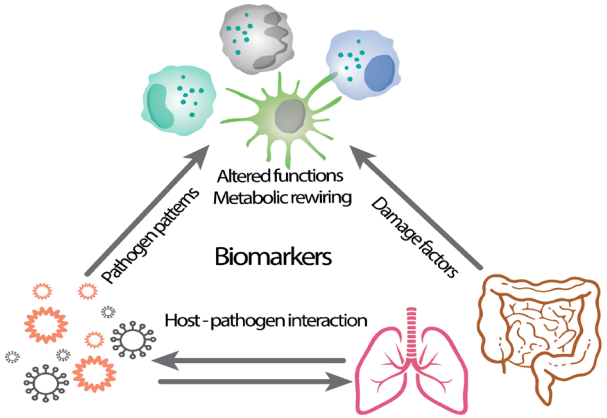
PROBLEMS & PARADIGMS

Prospects & Overviews

How immune-cell fate and function are determined by metabolic pathway choice

The bioenergetics underlying the immune response

Marcela Hortová-Kohoutková[1] | Petra Lázničková[1,2] | Jan Frič[1,3]



SHOCK, Vol. 54, No. 5, pp. 606–614, 2020

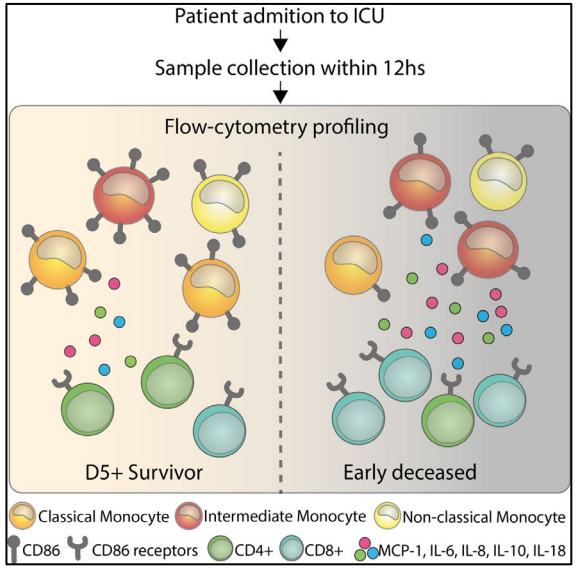
OPEN

SHOCK
Injury, Inflammation, and Sepsis: Laboratory and Clinical Approaches

Review Article

PHAGOCYTOSIS–INFLAMMATION CROSSTALK IN SEPSIS: NEW AVENUES FOR THERAPEUTIC INTERVENTION

Marcela Hortová-Kohoutková,* Federico Tidu,* Marco De Zuani,* Vladimír Šrámek,† Martin Helán,*† and Jan Frič*‡



BACKGROUND

Complexity of sepsis – central role of immune system

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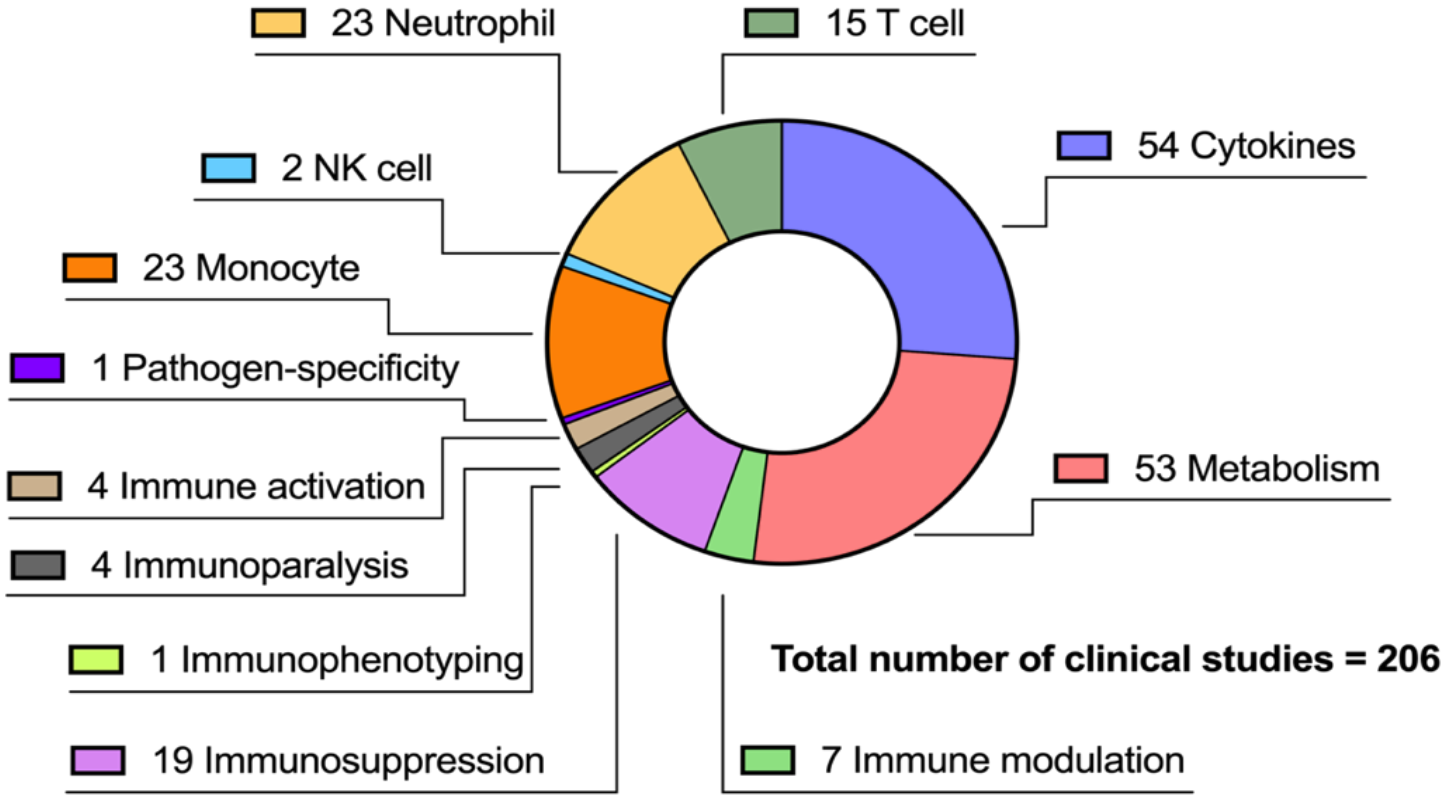
Fields of interest:

- Cytokines and other mediators
- Immune cells
  - neutrophils
  - monocytes
  - NK cells
- Adverse effects

Overview of active clinical studies targeting immune systém in sepsis



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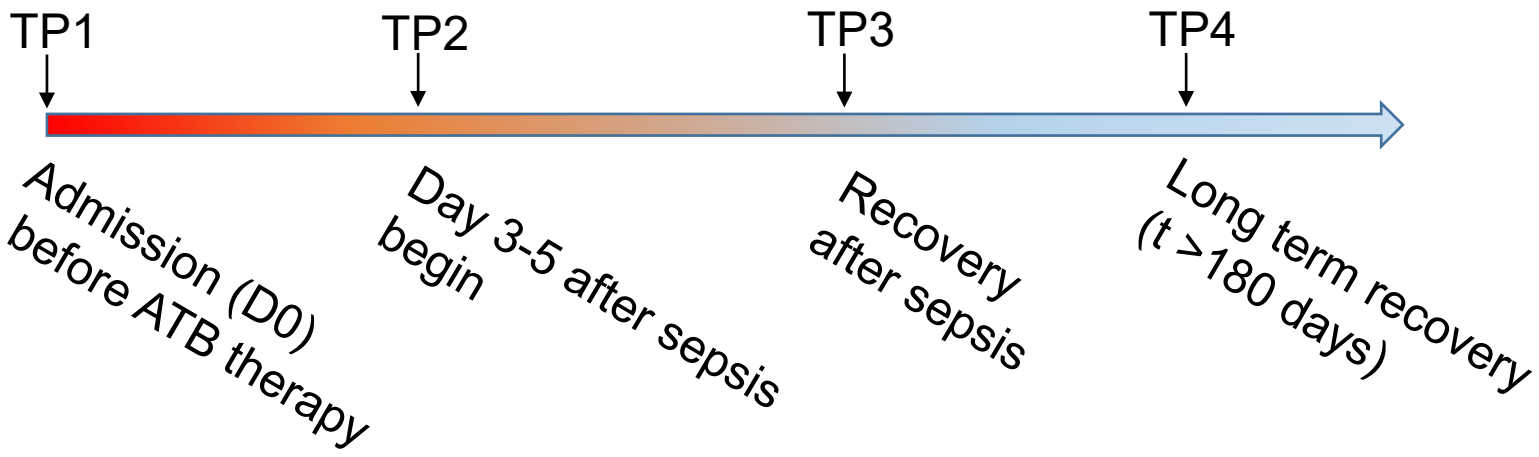
Immune markers of sepsis progression

Immune marker associated with sepsis	Best-practise diagnostics	"Experi-mentally obtained"	Markers used in different centers			
			Center #1	Center #2	Center #3	Center #4
Total WBC count	✓		✓	✓	✓	✓
CRP	✓		✓	✓	✓	✓
Procalcitonine	✓		✓	✓	✓	✓
Presepsin (sCD14)		✓	✓			✓
HLA-DR		✓	✓			
CD11b		✓		✓		
CD64		✓		✓		
CD69		✓		✓		
TREM		✓			✓	
IL-1		✓	✓	✓	✓	
IL-6		✓	✓	✓	✓	
TNF-α		✓		✓	✓	

PATIENT COHORTS

Sepsis, septic shock, febrile neutropenia



PATIENT COHORTS

Septic shock 41 patients (early diseased and early survivors)

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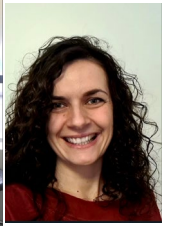
Demographic and clinical characteristics of patients with septic shock

Characteristic	Total	D5+ Survivors	Early deceased	*P* value
Recruited patients	41	33 (80.5%)	8 (19.5%)	—
Gender				
Female	17 (41.5%)	14 (82.4%)	3 (17.6%)	—
Male	24 (58.5%)	19 (79.2%)	5 (20.8%)	—
Age, mean (range)	71.3 (49-89)	70.6 (49-89)	74.1 (66-85)	.3850
Comorbidities, mean	2.23	2.22	2.25	.8253
BMI, mean	27.80	27.48	29.12	.3699
SOFA, mean	11.46	10.82	14.13	**.0360**
CRP mg/l, mean	224.32	213.38	268.06	.5388
Lactate mmol/l, mean	2.15	1.72	3.90	**.0045**
Origin of septic shock				
Pneumonia	17	17 (100%)	0 (0%)	—
Abdominal infection	7	5 (71.4%)	2 (28.6%)	—
Urosepsis	6	3 (50%)	3 (50%)	—
Soft tissue infection	5	3 (60%)	2 (40%)	—
Mediastinitis	3	3 (100%)	0 (0%)	—
Other	3	1 (33.3%)	2 (66.7%)	—

Abbreviations: BMI, body mass index; CRP, c-reactive protein; SOFA, sequential organ failure assessment.

Marcela Hortová Kohoutková

Petra Lázničková



Kamila Bendíčková

Journal of Cellular and Molecular Medicine

Differences in monocyte subsets are associated with short-term survival in patients with septic shock

Marcela Hortová-Kohoutková[1] | Petra Lázničková[1,2] | Kamila Bendíčková[1] | Marco De Zuani[1] | Ivana Andrejčinová[1,2] | Veronika Tomášková[1,3] | Pavel Suk[1,3] Vladimír Šrámek[3] | Martin Helán[1,3] | Jan Frič[1,4]

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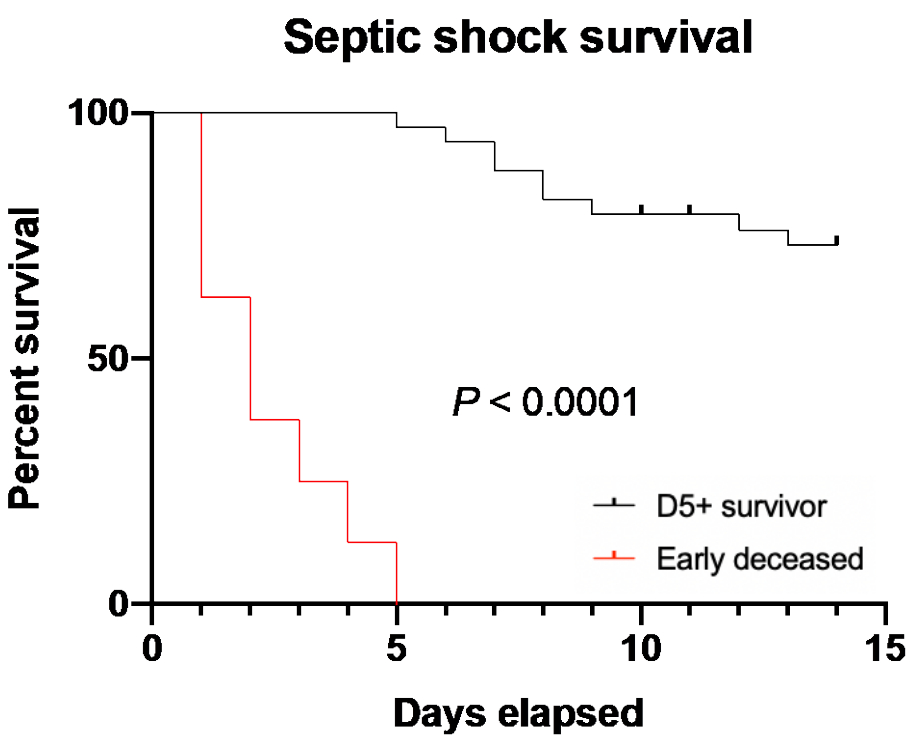
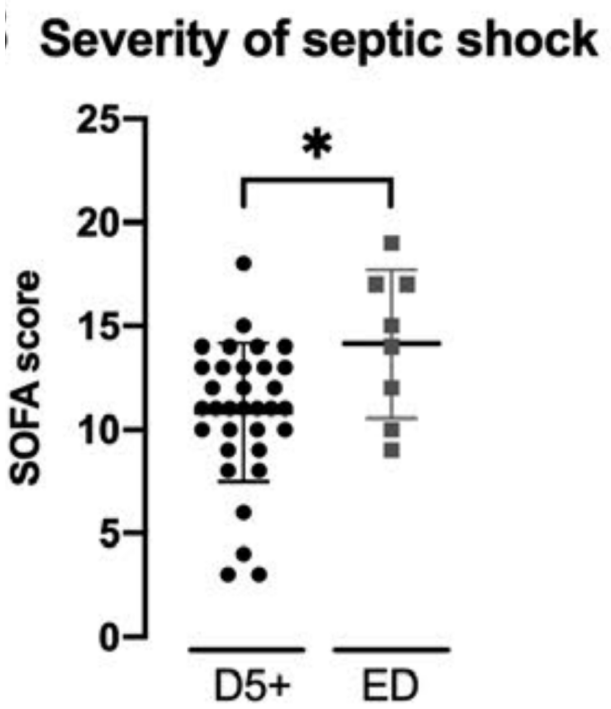
J Cell Mol Med. 2020;24:12504–12512.

PATIENT COHORTS

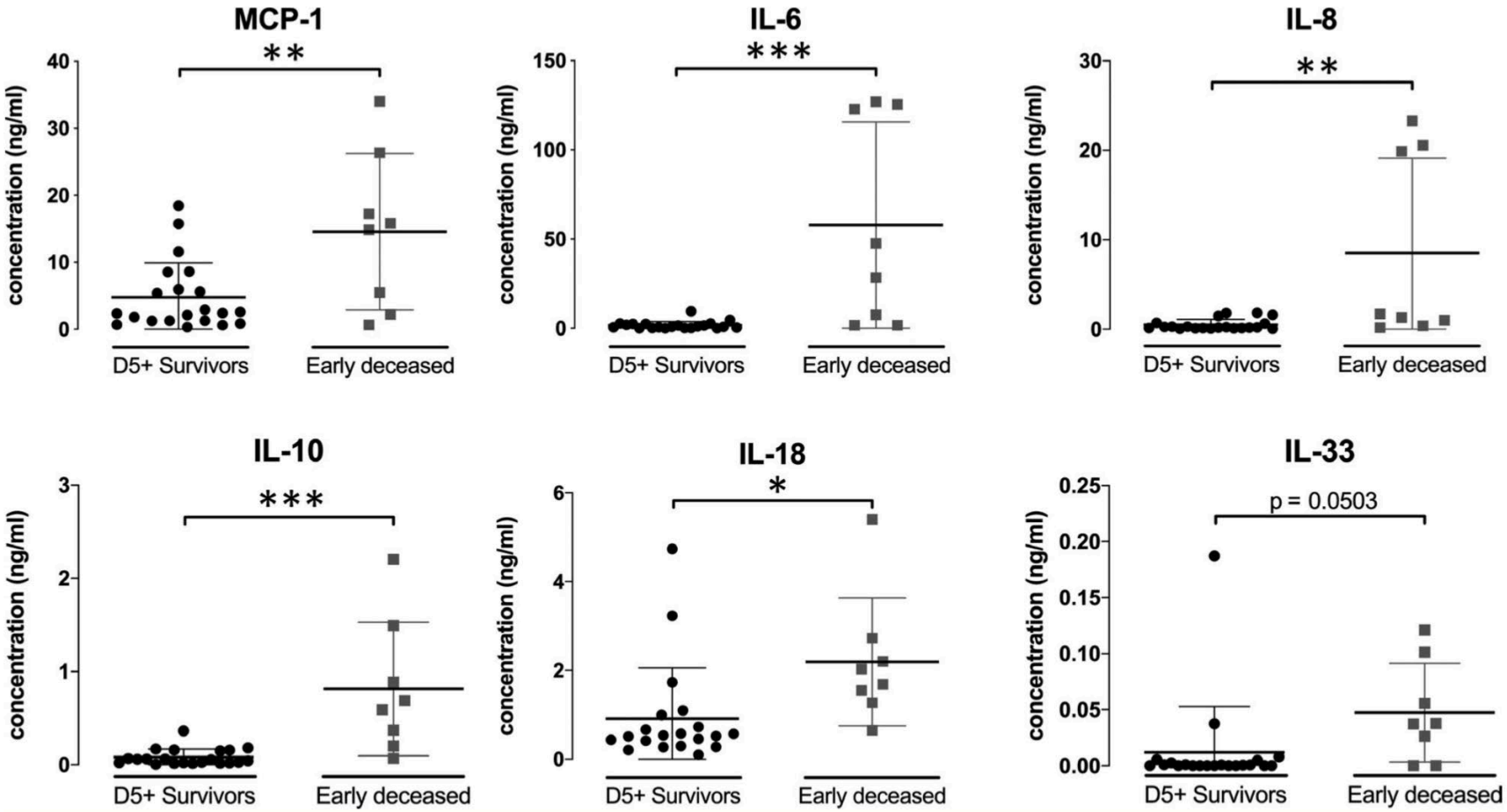
Septic shock 41 patients (early diseased and early survivors)





RESULTS

Monocytes in patients with septic shock



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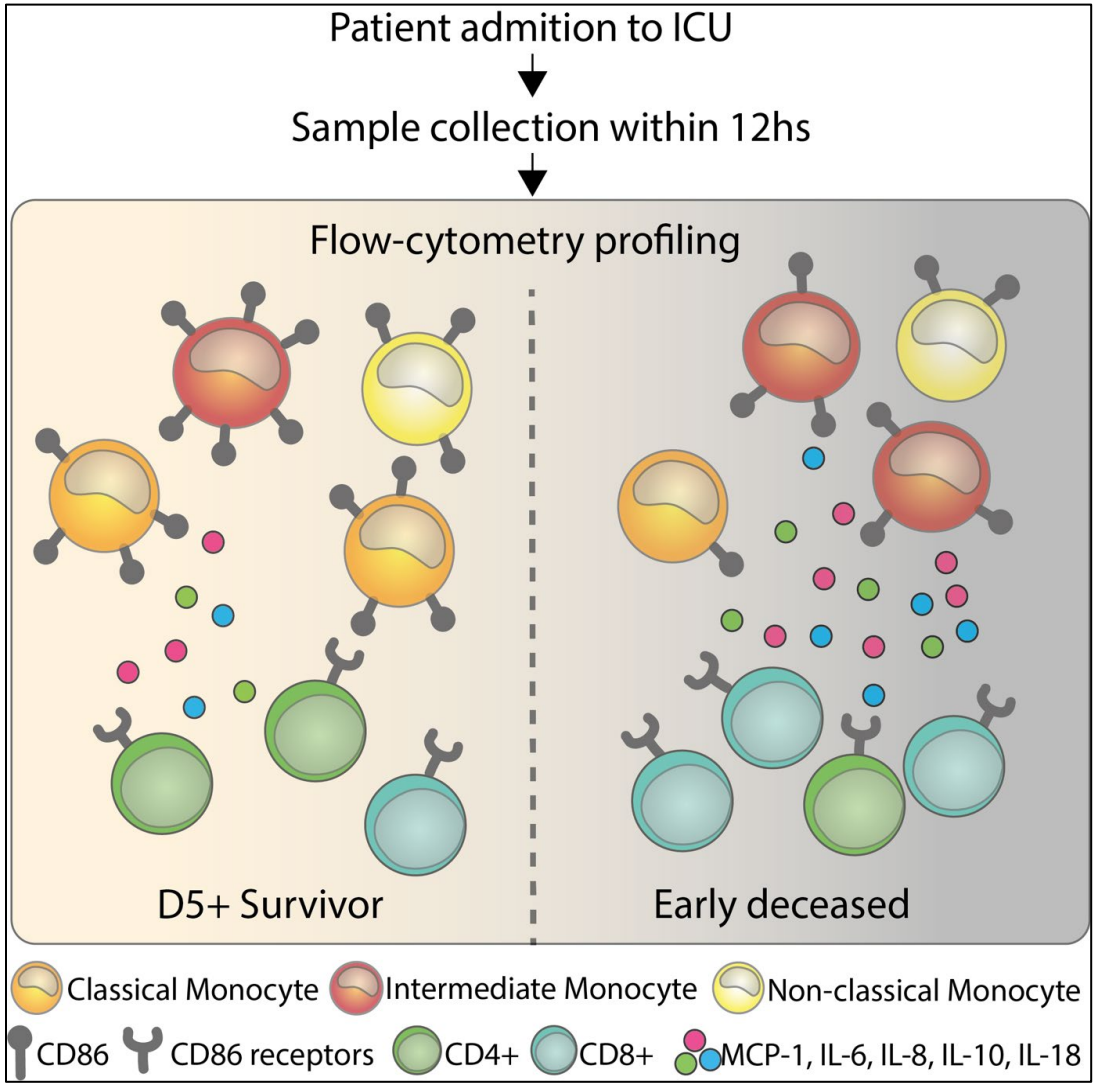

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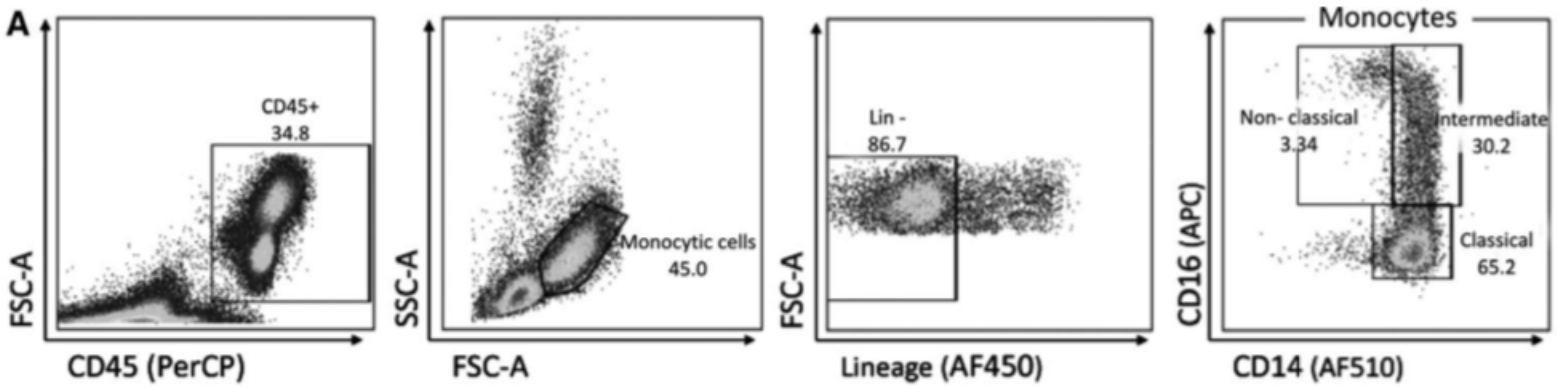
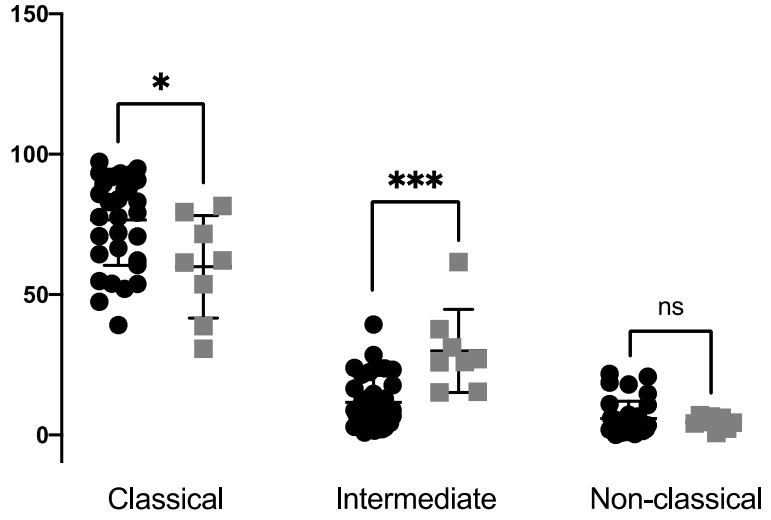
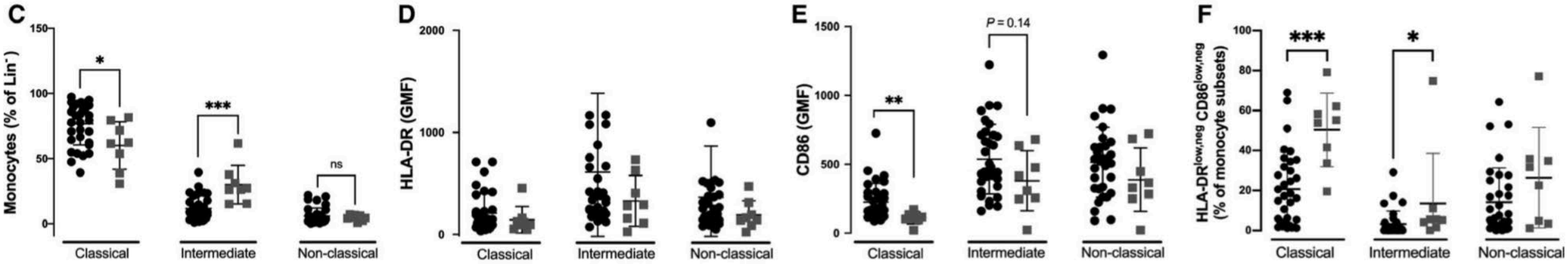




RESULTS

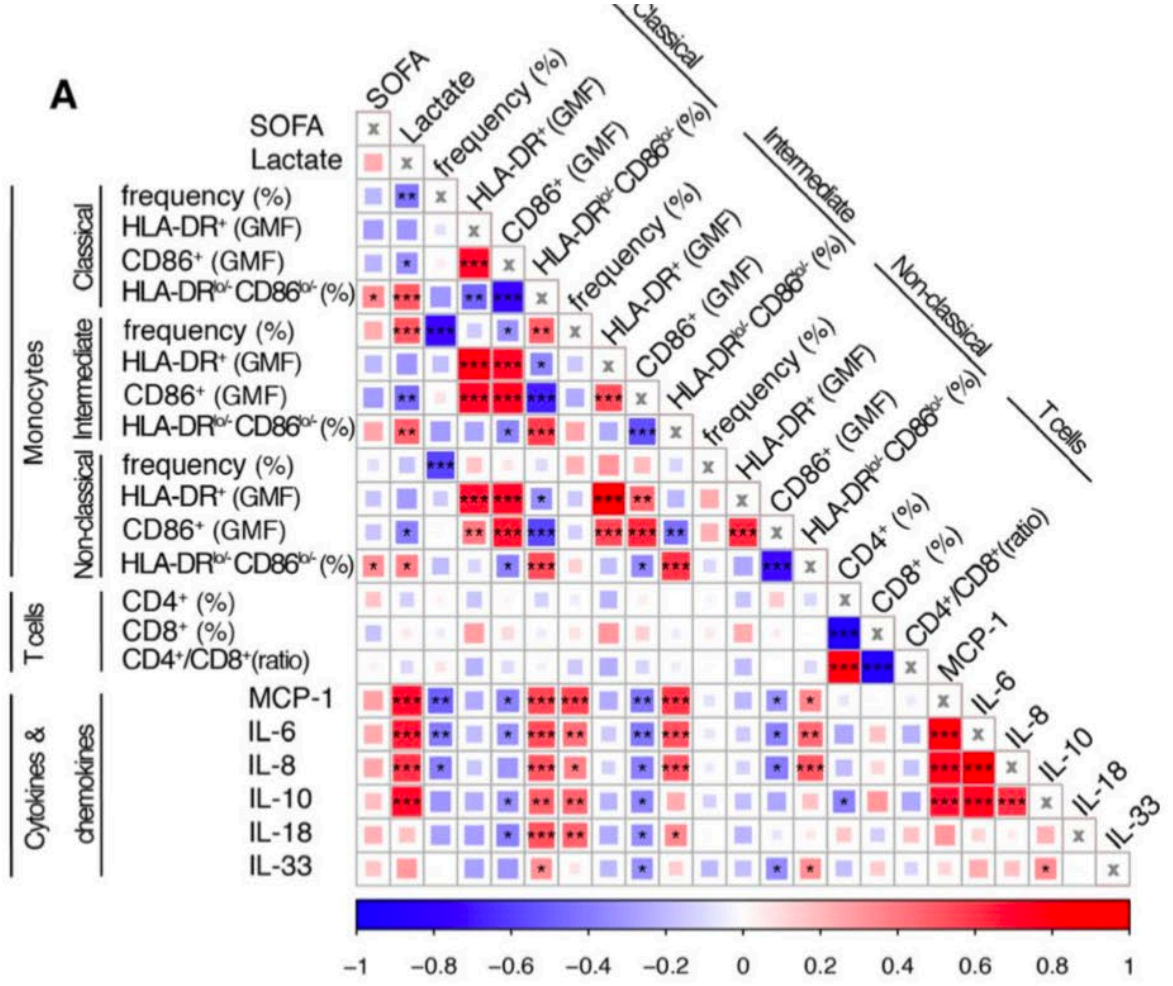
Monocytes in patients with septic shock

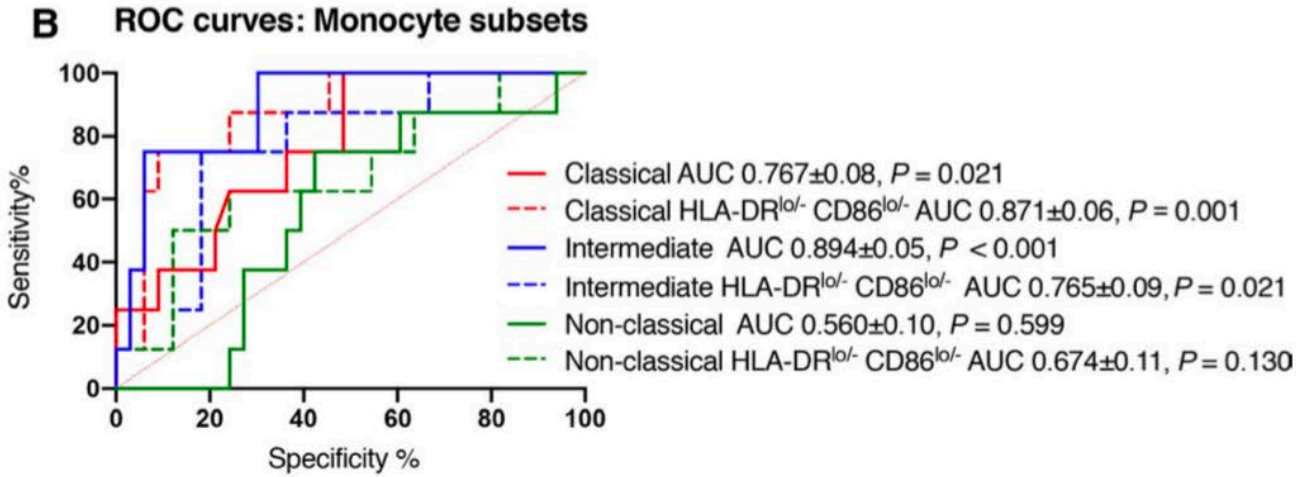


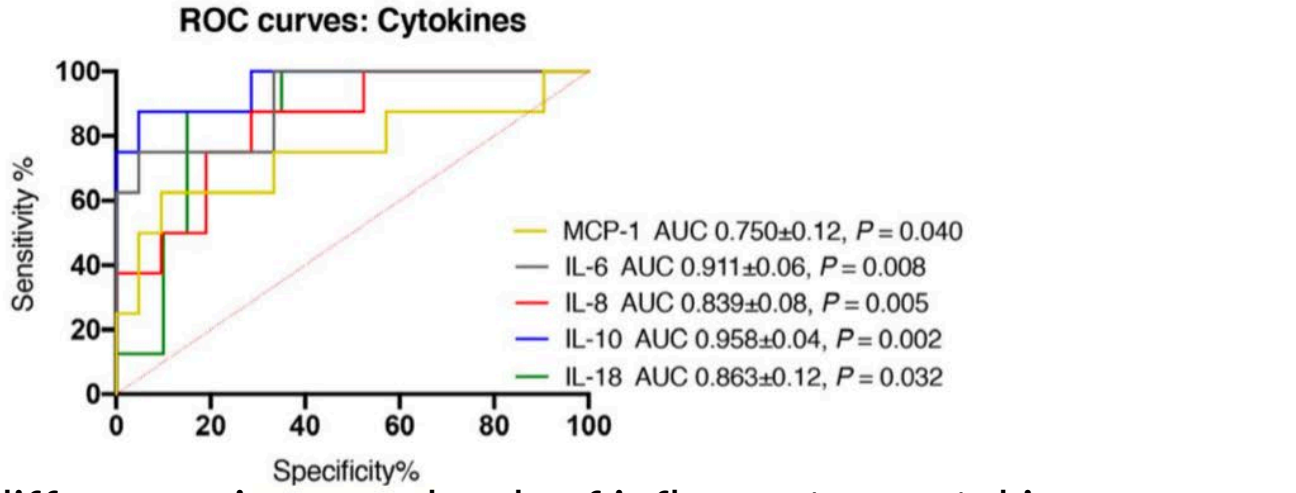
Frequency of classical and intermediate monocytes assessed at the time of admission to the intensive care unit are significantly distinct in patients with septic shock who survived longer that five days from those who died.

RESULTS

Monocytes in patients with septic shock



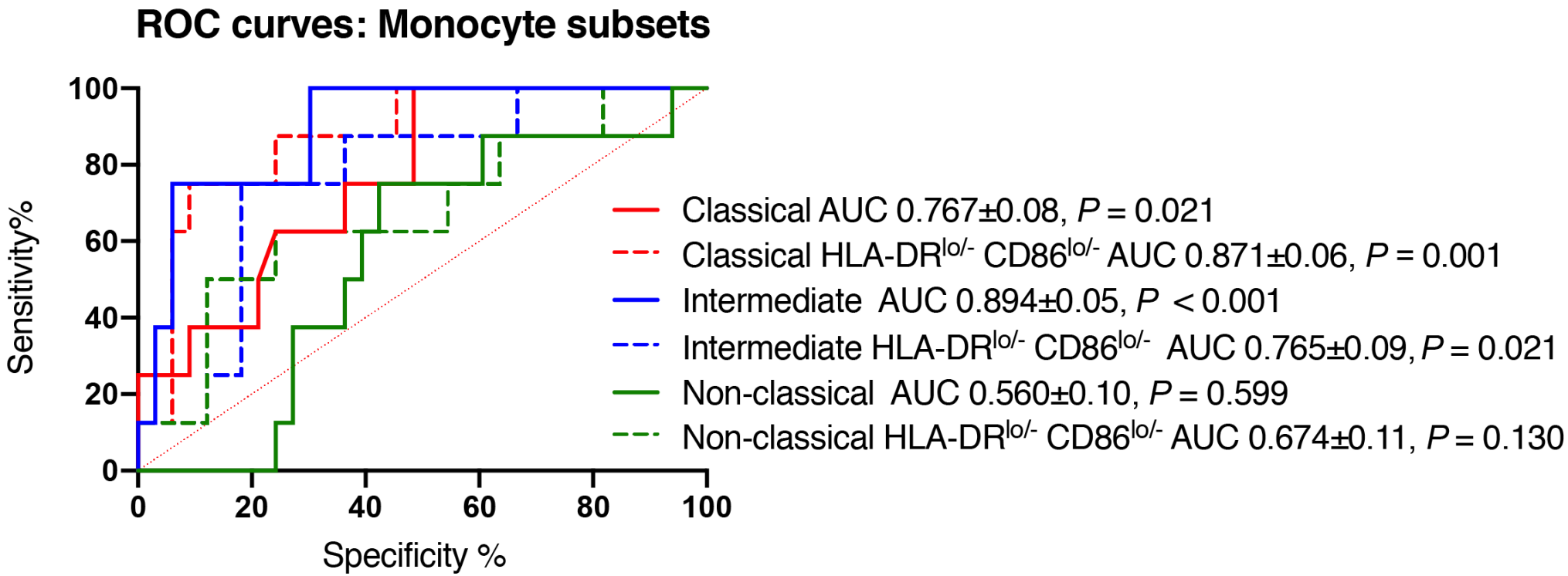




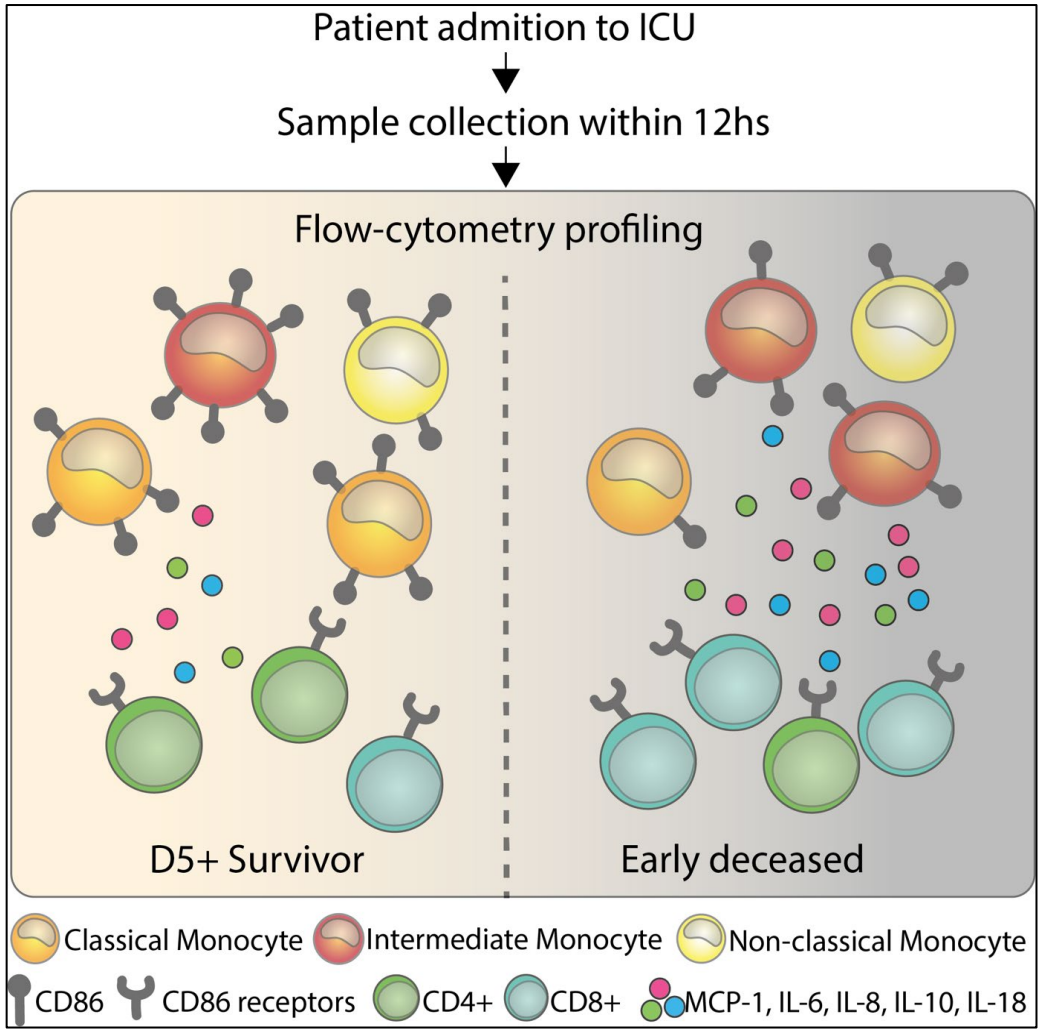
The changes in monocytes correlate significantly with differences in serum levels of inflammatory cytokines MCP-1, IL-6, IL-8, IL-10, and IL-18.

RESULTS

The diagnostic ability of monocyte changes – ROC curve



CONCLUSION



Hortová-Kohoutková et al., JCMM 2020

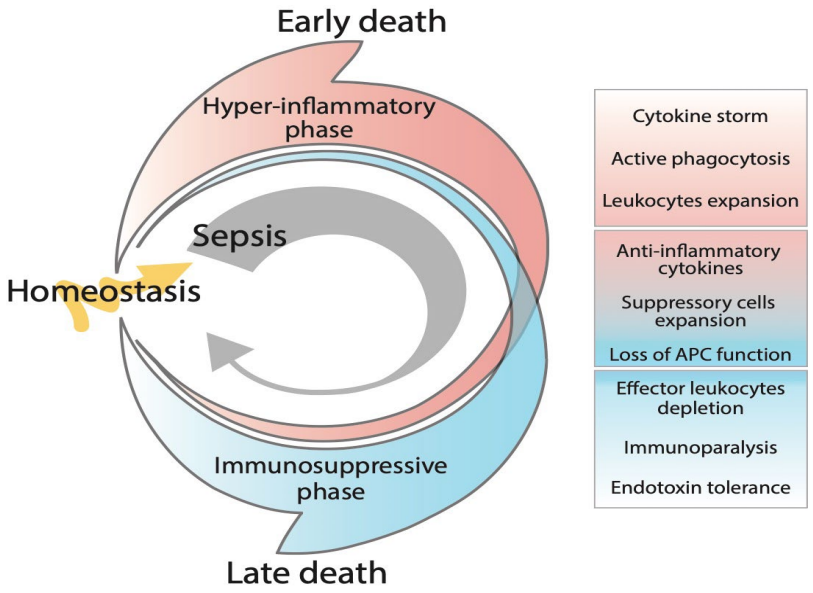
Monocyte subset frequency:

- **predictive marker** of septic shock survival
- **independent on stimuli** inducing septic shock

Monocytes activation status (CD86) significantly reduced

GOAL:

Advantage of timely cytometric analysis for identification of high risk group of patinets and ev. non - responders to conventional therapy



Hortová-Kohoutková et al., Shock 2020

The role of metabolism and transcription factor networks in myeloid cells and progression of human sepsis

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Journal of Cellular and Molecular Medicine

Differences in monocyte subsets are associated with short-term survival in patients with septic shock

Marcela Hortová-Kohoutková[1] | Petra Lázničková[1,2] | Kamila Bendíčková[1] | Marco De Zuani[1] | Ivana Andrejčinová[1,2] | Veronika Tomášková[1,3] | Pavel Suk[1,3] Vladimír Šrámek[3] | Martin Helán[1,3] | Jan Frič[1,4]

J Cell Mol Med. 2020;24:12504–12512.

European Journal of Immunology — Immunity to infection

Short Communication

Human myeloid-derived suppressor cell expansion during sepsis is revealed by unsupervised clustering of flow cytometric data

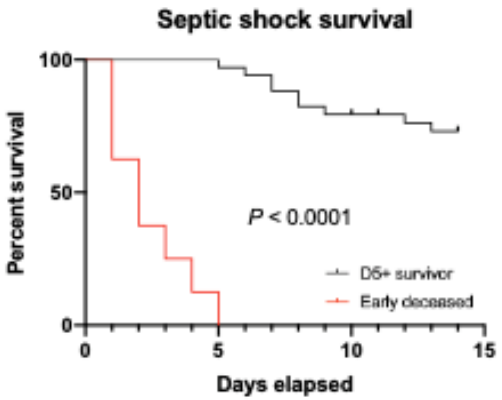
Marco De Zuani[1], Marcela Hortová-Kohoutková[1], Ivana Andrejčinová[1,2], Veronika Tomášková[1,3], Vladimír Šrámek[3], Martin Helán[1,3] and Jan Frič[1,4]

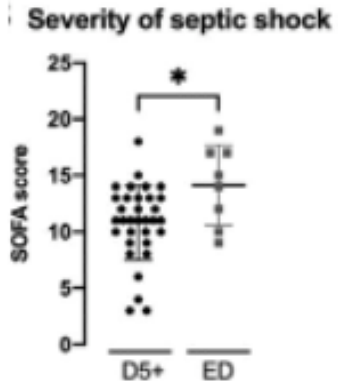
frontiers in Immunology

ORIGINAL RESEARCH
published: 13 December 2021
doi: 10.3389/fimmu.2021.741484

Polymorphonuclear Cells Show Features of Dysfunctional Activation During Fatal Sepsis

Marcela Hortová-Kohoutková[1†], Marco De Zuani[1†], Petra Lázničková[1,2], Kamila Bendíčková[1], Ondřej Mrkva[1], Ivana Andrejčinová[1,2], Alexandra Mýtniková[1], Ondřej Polanský[1], Kamila Kočí[1], Veronika Tomášková[3], Vladimír Šrámek[3], Martin Helán[1,3] and Jan Frič[1,4]*

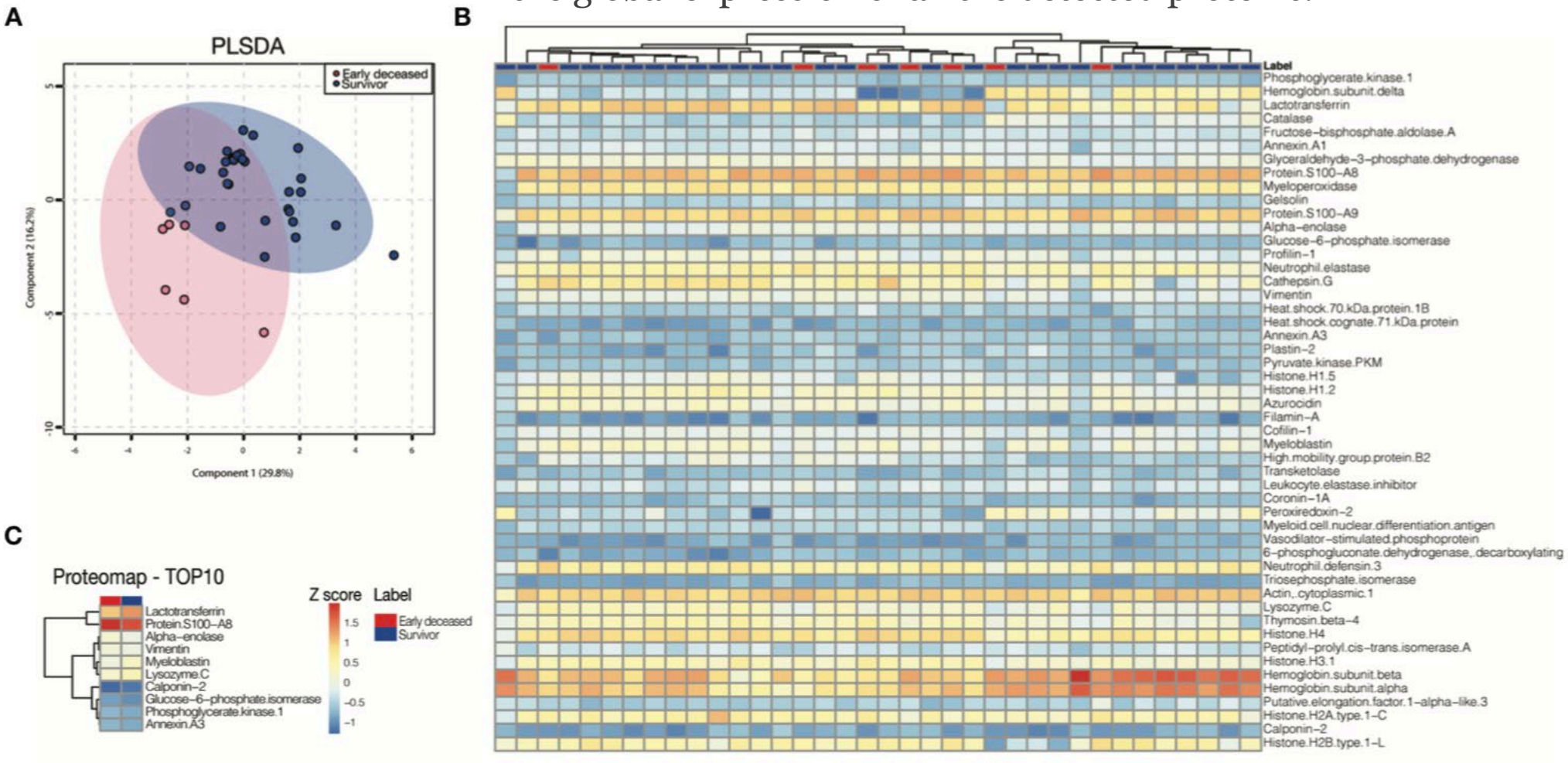




Profound and longterm changes of myeloid cells and their functions during sepsis

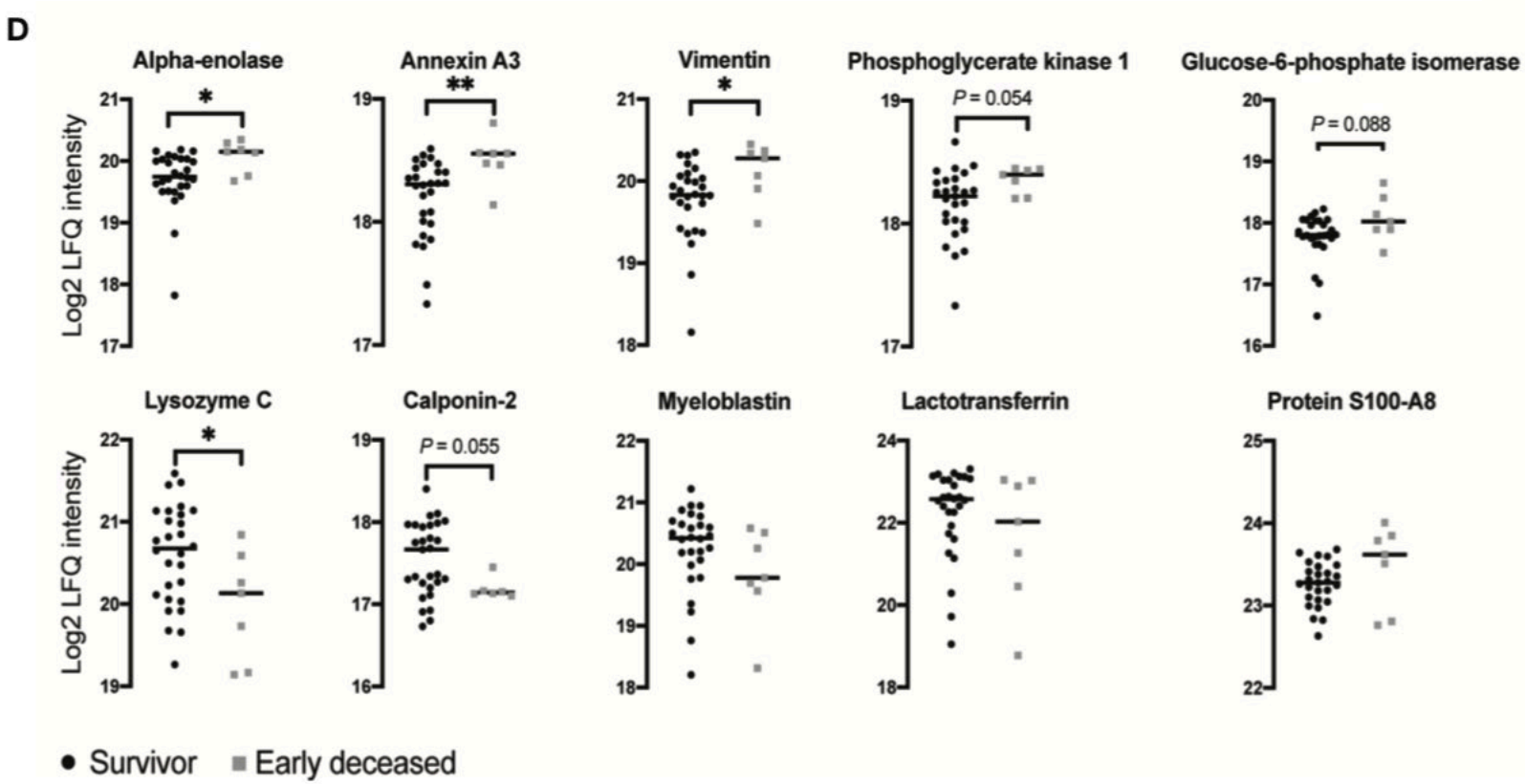
Overview and analysis of proteomics data.

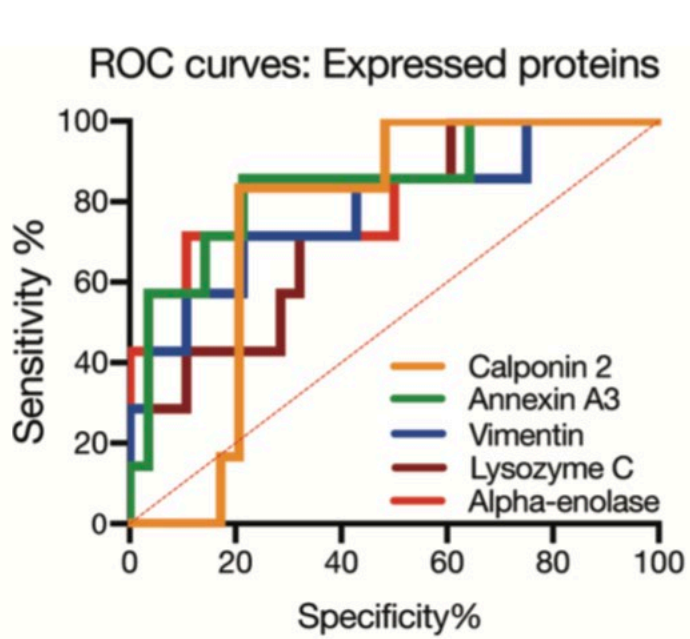
the global expression of all the detected proteins.



Polymorphonuclear Cells Show Features of Dysfunctional Activation During Fatal Sepsis

The LFQ intensities of the top 10 proteins were quantified and showed significant changes in alpha-enolase, Annexin A3, Vimentin, and Lysozyme





Term	AUC	*P* value	Std. error
Alpha-enolase	0.808	**0.013**	0.070
Annexin A3	0.810	**0.018**	0.101
Vimentin	0.781	**0.023**	0.106
Lysozyme C	0.759	**0.036**	0.093
Calponin 2	0.753	0.054	0.084
Phosphoglycerate kinase	0.724	0.070	0.092
Glucose-6-phosphate isomerase	0.725	0.070	0.116
S100-A8	0.689	0.127	0.159
Myeloblastin	0.679	0.145	0.102
Lactotransferrin	0.636	0.272	0.118

Polymorphonuclear Cells Show Features of Dysfunctional Activation During Fatal Sepsis

Predictive potential of dynamic changes in neutrophil and monocyte subsets in SIRS and sepsis development after surgery or trauma.

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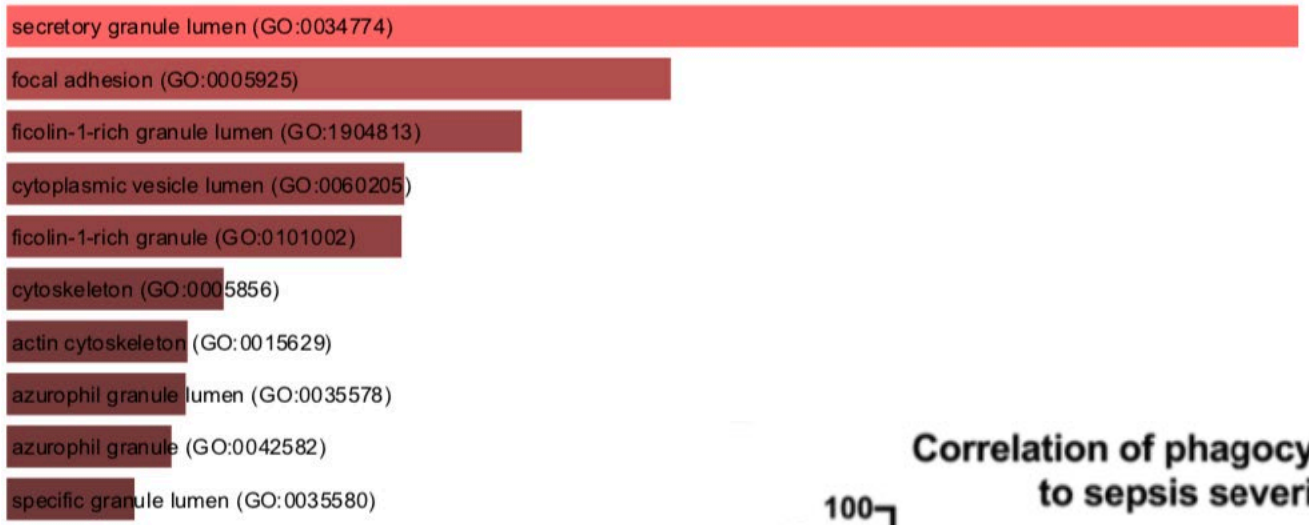

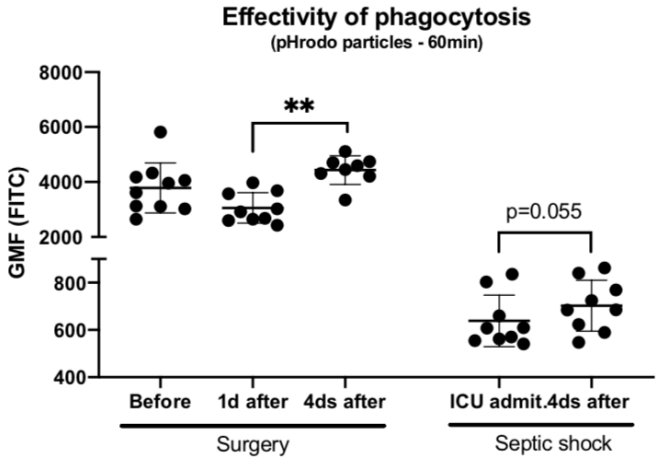

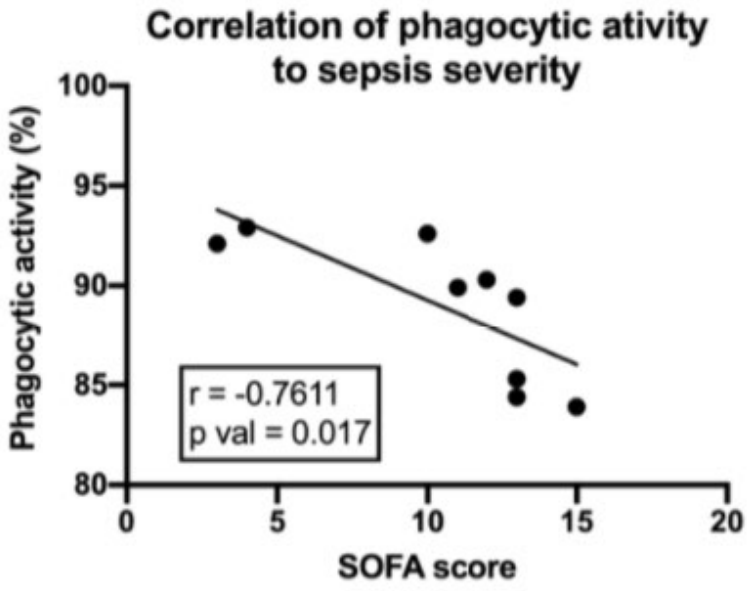

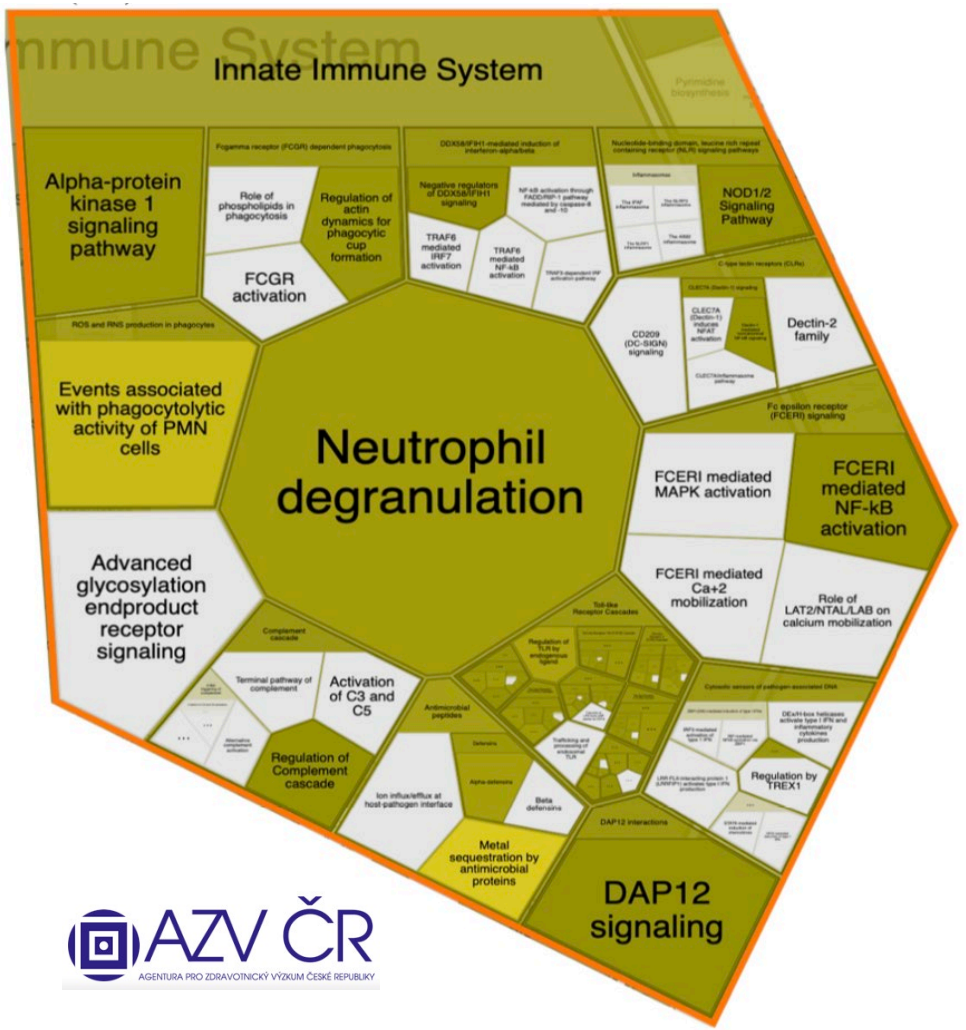

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Outline

- the importance of the immune system in sepsis
- why sepsis research @FricLab
- diagnostics markers from the immune system
- therapeutic targets originated in the immune system
- **Adverse effects of sepsis - trained immunity**

Metabolic reprogramming and innate memory

frontiers in Immunology

ORIGINAL RESEARCH
published: 13 December 2021
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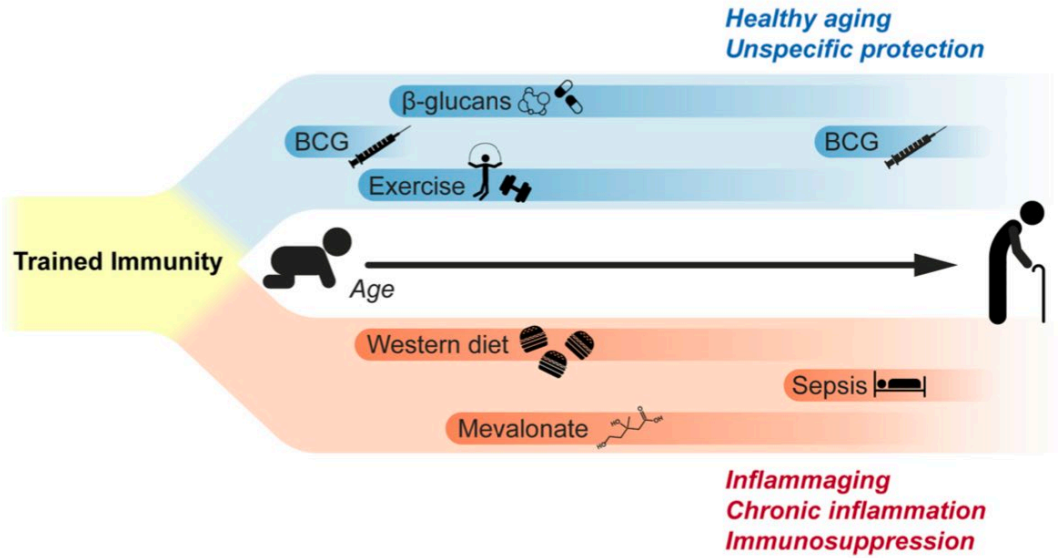
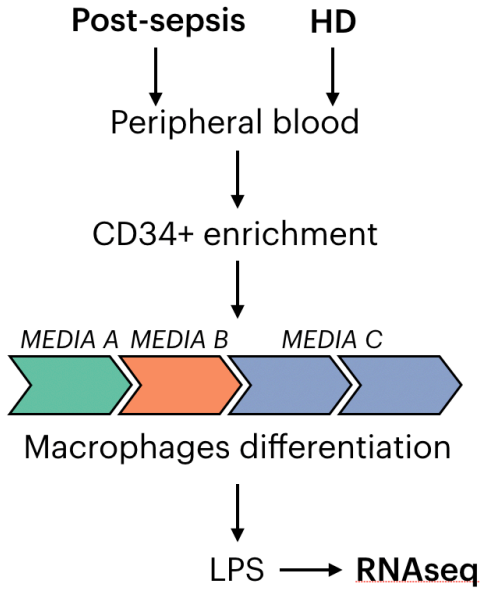
Train the Trainer: Hematopoietic Stem Cell Control of Trained Immunity

Marco De Zuani[1] *and Jan Frič*[1,2*]

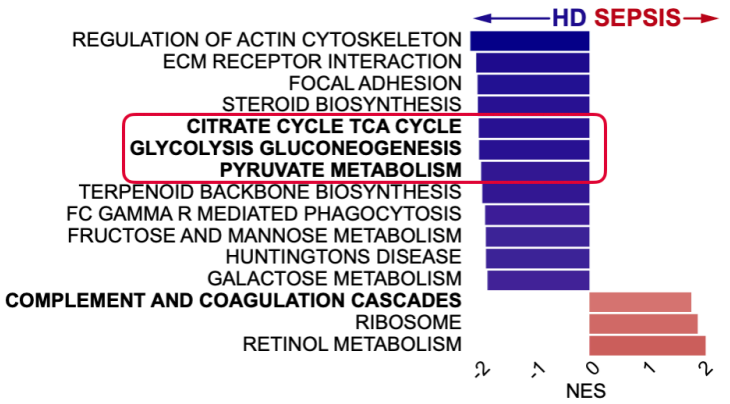
[1] International Clinical Research Center, St. Anne's University Hospital, Brno, Czechia, [2] Institute of Hematology and Blood Transfusion, Prague, Czechia



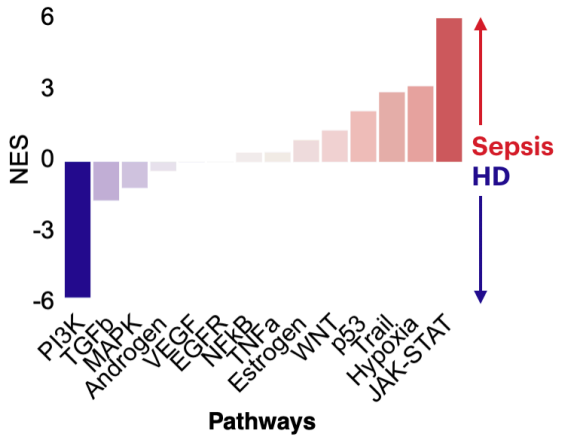
HSDM transcriptome profiling



Gene-set enrichment analysis (GSEA)



Macrophages derived from **post-sepsis** HSCs are **metabolically impaired**



„Trainned immunity“ – význam u nových pandemií?

Paměť vrozené imunity jako nástroj pro obranu organismu před mikrobiální a SARS-CoV-2 pneumonií se závažným průběhem

- *Trainned immunity* – tzv. vyškolená imunita
- Antigenní stimulace vede ke vzniku paměťových buněk
- Poskytuje zkříženou ochranu proti různým patogenům

ORIGINAL ARTICLE

Scandinavian Journal of Immunology WILEY

High CD4-to-CD8 ratio identifies an at-risk population susceptible to lethal COVID-19

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- Podstatou je epigenetické a metabolické přeprogramování buněk vrozené imunity a jejich hematopoetických prekurzorů
- Možná aktivace očkováním

Cílená aktivace „trainned immunity“ (např. pomocí BCG vakcinace) by mohla mít ochranou roli při vzniku nových pandemií, než bude vyvinuta specificky cílená vakcína.

Myeloid derived suppressor cells expansion in septic shock and in long-term sepsis survivors

FNUSA ICRC

Marco
De Zuani

Myeloid derived suppressor cells (MDSCs):

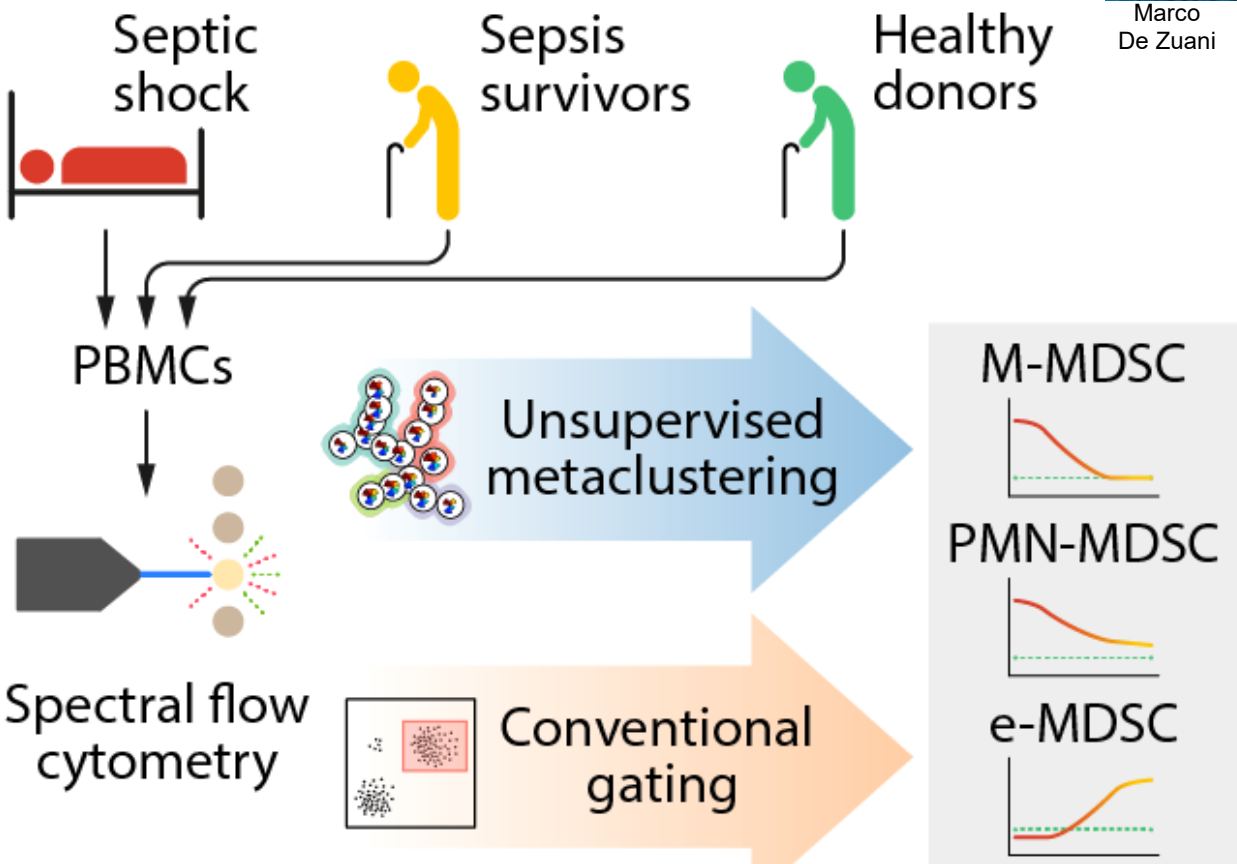
- heterogeneous population of immature myeloid cells
- strong immunosuppressive activity, especially on T cells and NK cells
- low frequencies in healthy donors, but rapidly expand in pathological conditions (cancer, infections, …)
- a pathologic role by supressing the protective immune response
- few studies reported that MDSC expansion might actually be beneficial, restraining potentially damaging inflammation - **dual role of MDSCs in sepsis**
- **Roles in acute sepsis as well as in the long-term complications seen in survivors.**

Short Communication | Clinical | Free Access

European Journal of Immunology
Basic · Clinical · Translational

Human myeloid-derived suppressor cell expansion during sepsis is revealed by unsupervised clustering of flow cytometric data

Marco De Zuani, Marcela Hortová-Kohoutková, Ivana Andrejčinová, Veronika Tomášková, Vladimír Šrámek, Martin Helán, Jan Frič



De Zuani et al.,Eur J Immunol 2021

Myeloid derived suppressor cells expansion in septic shock and in long-term sepsis survivors

FNUSA ICRC

Table 1: Cohort characteristics

Study cohort	n	12
Sex	Female	6 (50%)
	Male	6 (50%)
Age, mean (range)		64.75 (28-77)
Comorbidities, mean		3.25
	Hypertension, n	7 (58.3%)
	Peripheral artery disease, n	4 (33.3%)
	Asthma, n	3 (25%)
	Coronary heart disease, n	4 (33.3%)
BMI [kg/m^2], mean		27.24
Mortality	n	5 (41.7%)
Causative agent	Soft tissue infection, n	1 (8.3%)
	Urosepsis, n	5 (41.7%)
	Mediastinitis / empyema, n	2 (16.7%)
	Pneumonia, n	2 (16.7%)
	Unknown, n	2 (16.7%)
TP1	SOFA, mean	13.3
	CRP [mg/l], mean	240.41
	Leucocyte count [10^9/l], mean	22.23
	Horowitz index, mean	204.84
	Noradrenalin dose [ug/kg/min], mean	0.382
TP2	SOFA, mean,	5.9
	CRP [mg/l], mean	81.88
	Leucocyte count [10^9/l], mean	16.26
	Horowitz index, mean	295.65
	Noradrenalin dose [ug/kg/min], mean	0.01
Post-sepsis cohort	n	6
Sex	Female	4 (66%)
	Male	2 (33%)
Age, mean (range)		69.50 (60-76)
Comorbidities, mean		2.17
BMI [kg/m^2], mean		28.78
Data at ICU admission	SOFA, mean	10.00
	CRP [mg/l], mean	386.78
	Leucocyte count [10^9/l], mean	8.767
	Horowitz index, mean	218.04
	Noradrenalin dose [ug/kg/min], mean	0.09
Causative agent	Abdominal infection, n	2 (33.3%)
	Pneumonia, n	1 (16.7%)
	Mediastinitis / empyema, n	2 (33.3%)
	Combined, n	1 (16.7%)
follow-up	Leucocyte count [10^9/l], mean	6.12
Control Cohort	n	7
Sex	Female	4 (57%)
	Male	3 (43%)
Age, mean (range)		70.8 (63-82)
Comorbidities, mean		2.4
BMI [kg/m^2], mean		29.79
CRP [mg/l], mean		2.75

Multidimensional flow cytometry clustering demonstrates MDSCs expansion during septic shock and in long-term survivors

Marco De Zuani

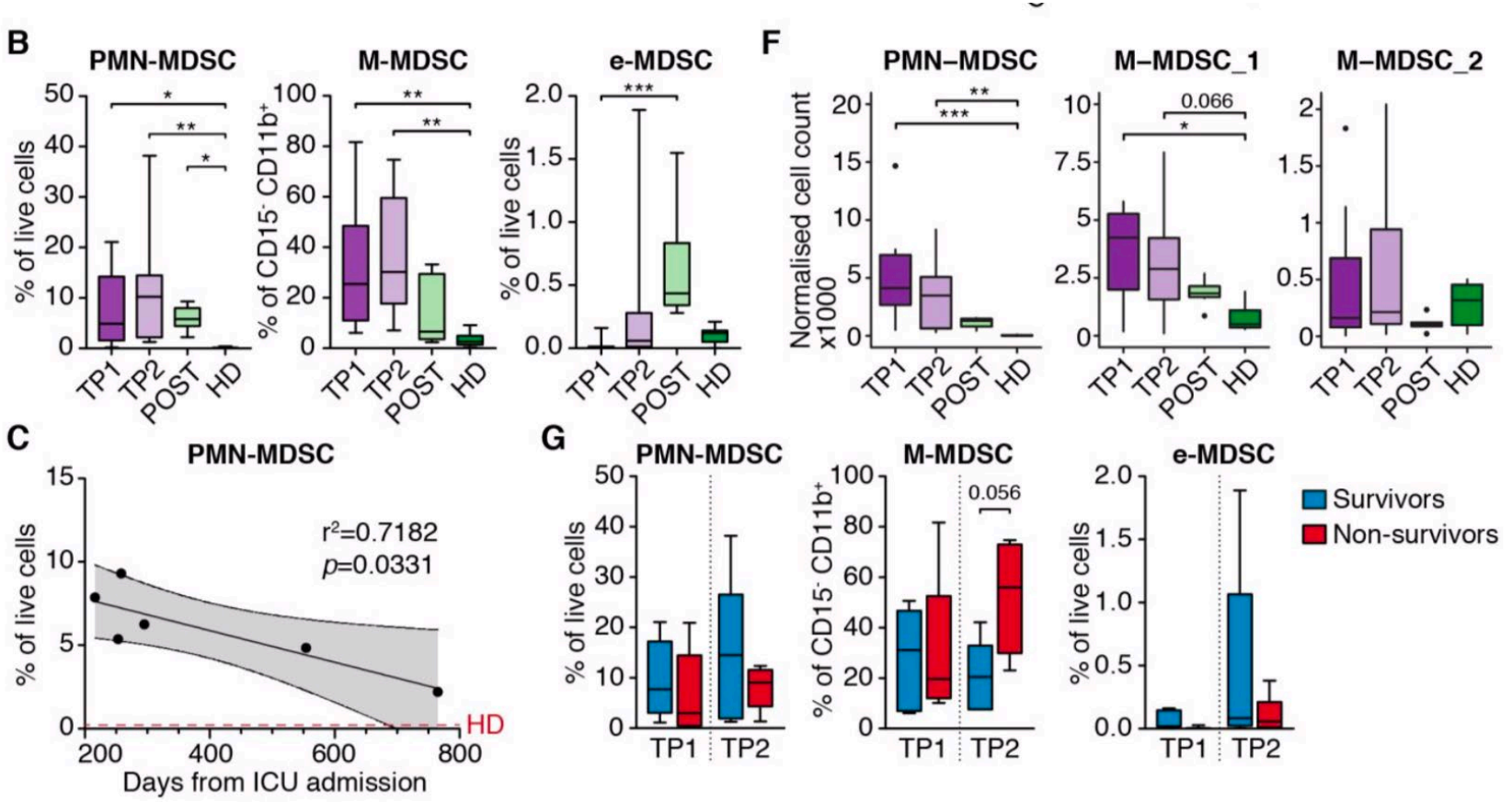
Study overview:

- combined comprehensive flow cytometry phenotyping with unsupervised clustering using self-organizing maps

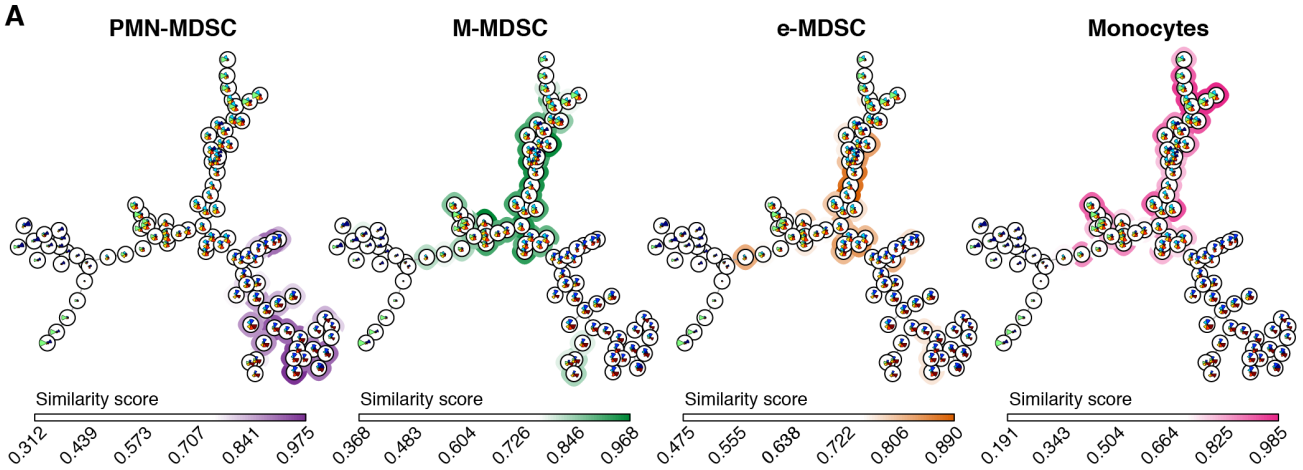
MDSC subsets in blood:
- myeloid - M-MDSCs
- polymorphonuclear PMN-MDSCs
- early-stage (e)-MDSCs

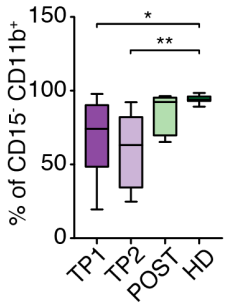
- severe sepsis patients
- long-term sepsis survivors
- age-matched controls

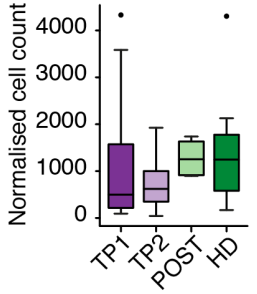
Circulating HSPCs during sepsis

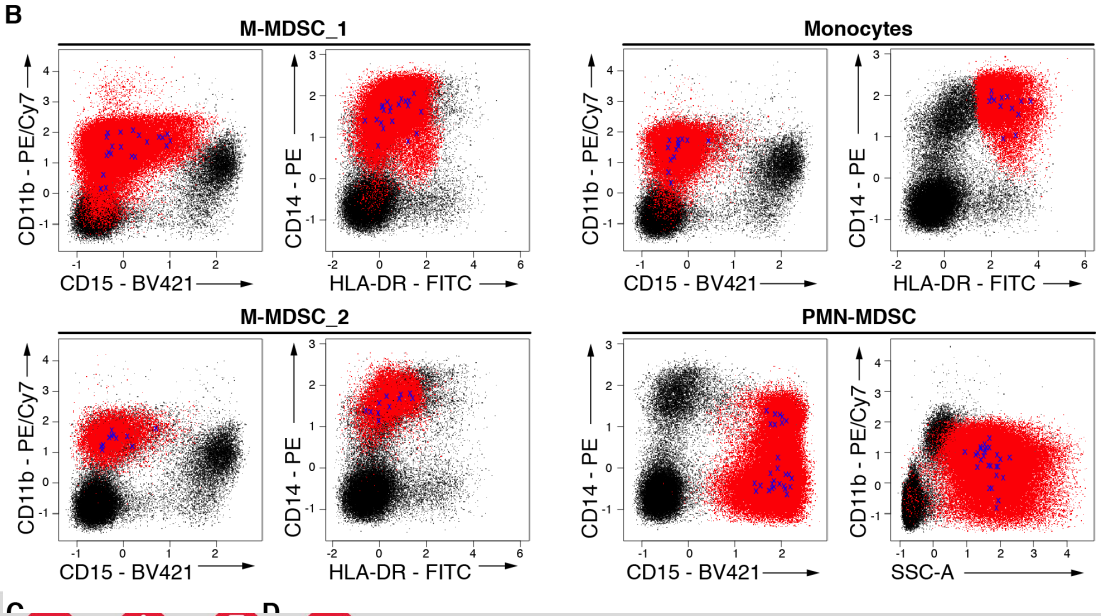


Results - Unsupervised clustering confirms M-MDSC and PMN-MDSC expansion during sepsis









- The background intensity of the nodes in each minimum spanning tree plot corresponds to the degree of similarity with the training profile. Nodes with an “identity score” higher than 0.9 were used to identify the main metaclusters.
- To confirm whether the identifier metaclusters were phenotypically concordant with those of the manually-gated cells, the events in each metacluster were gated on the representative 2Dplots used for manually gating the MDSCs populations.

CONCLUSION

Marco De Zuani

- we demonstrate that both M-MDSCs and PMN-MDSCs but not e-MDSCs are present at high levels in patients with early-stage sepsis
- high levels of PMN-MDSCs were also present in long-term survivors many months after discharge, suggesting a possible role in sepsis-related complications.
- employing unsupervised clustering of flow cytometric data we have confirmed the likely involvement of human MDSC subsets **in acute sepsis**, and revealed their expansion in sepsis survivors at **late timepoints**.
- the application of this strategy in future studies and in the clinical/diagnostic context would enable rapid progress towards a full understanding of the roles of MDSC in sepsis and other inflammatory conditions.

CENTER FOR TRANSLATIONAL MEDICINE

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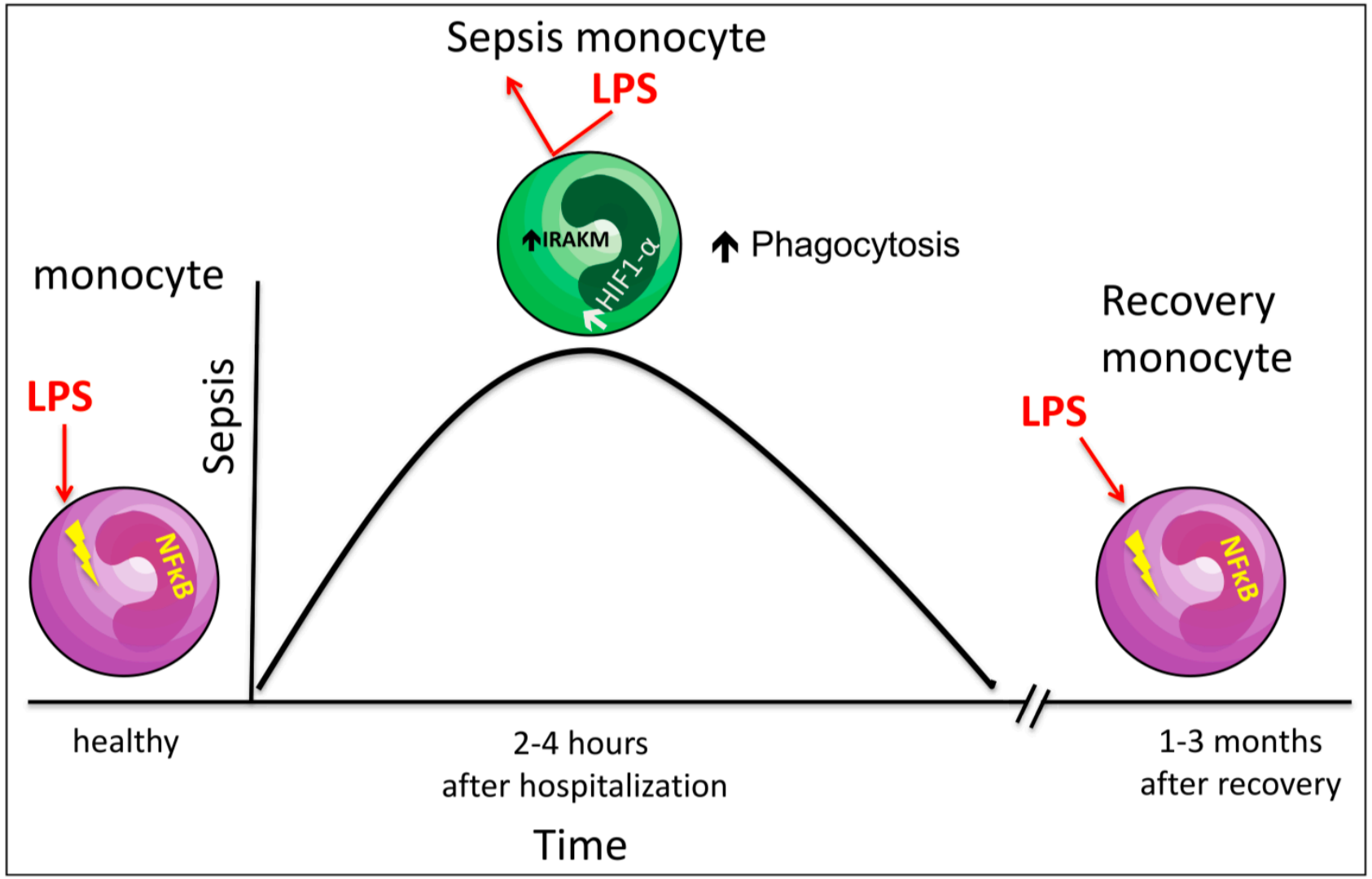
MŠMT · OPVVV · AZV ČR Agentura pro zdravotnický výzkum České republiky · Interreg Österreich-Tschechische Republik Europäischer Fonds für regionale Entwicklung · European Commission · MARIE CURIE ACTIONS

BACKGROUND

Sepsis induced monocyte plasticity



Central role of monocytes in sepsis:

- dynamic expression changes
- reporogramming
- immunosupressive phenotype
- long term -endotoxin tolerance

BACKGROUND
The breakthrough of modern therapies

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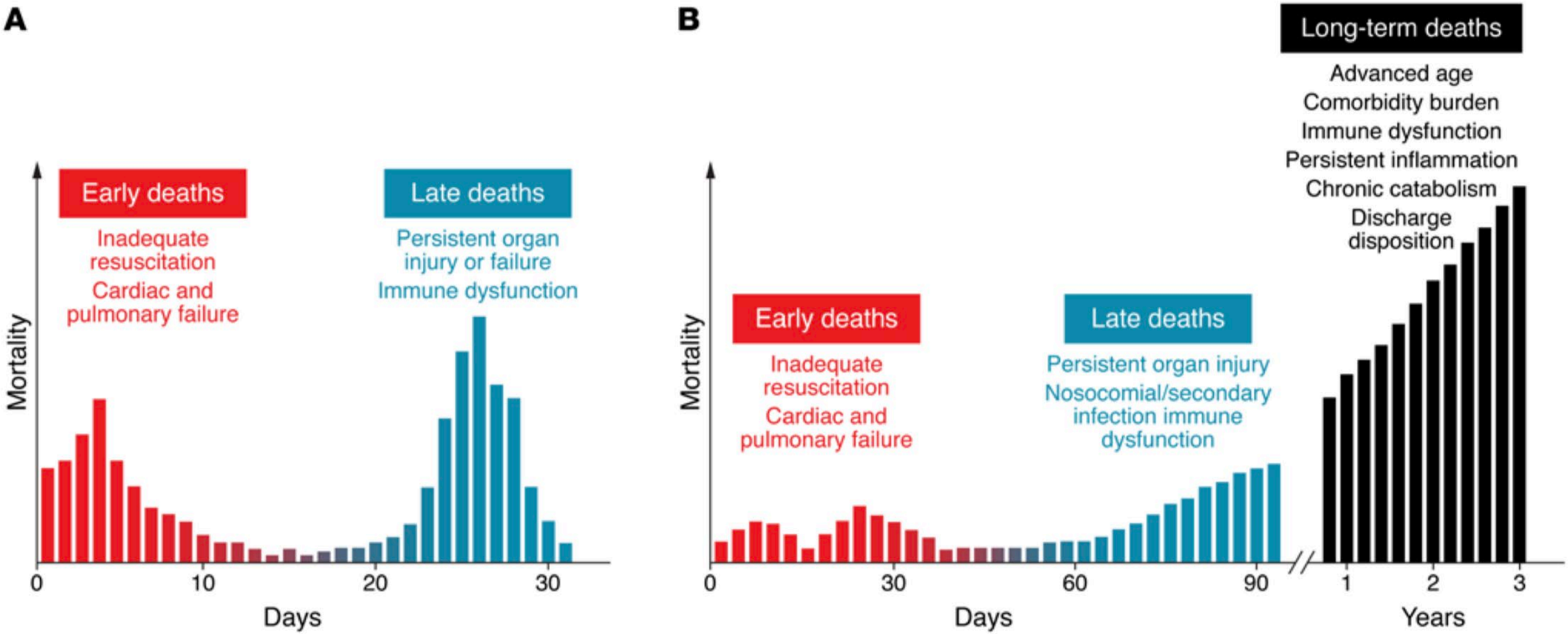
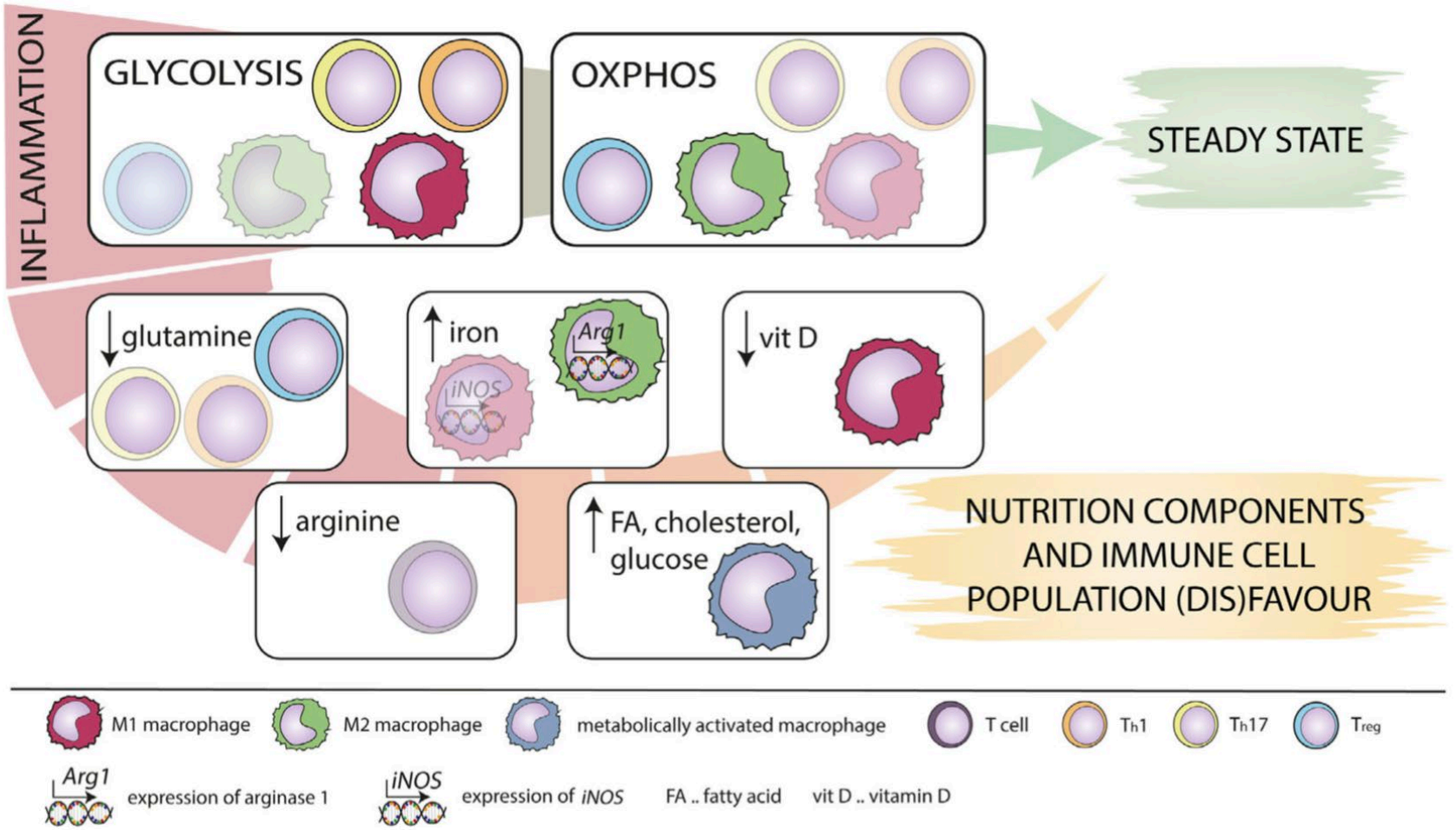


Immune cells functions are connected to immunometabolism

Hortova-Kohoutkova M, Lázničková P, Frič J, Bioessays 2021



T_h1 and T_h17

- high rate of glycolysis

Treg

- predominantly use OXPHOS

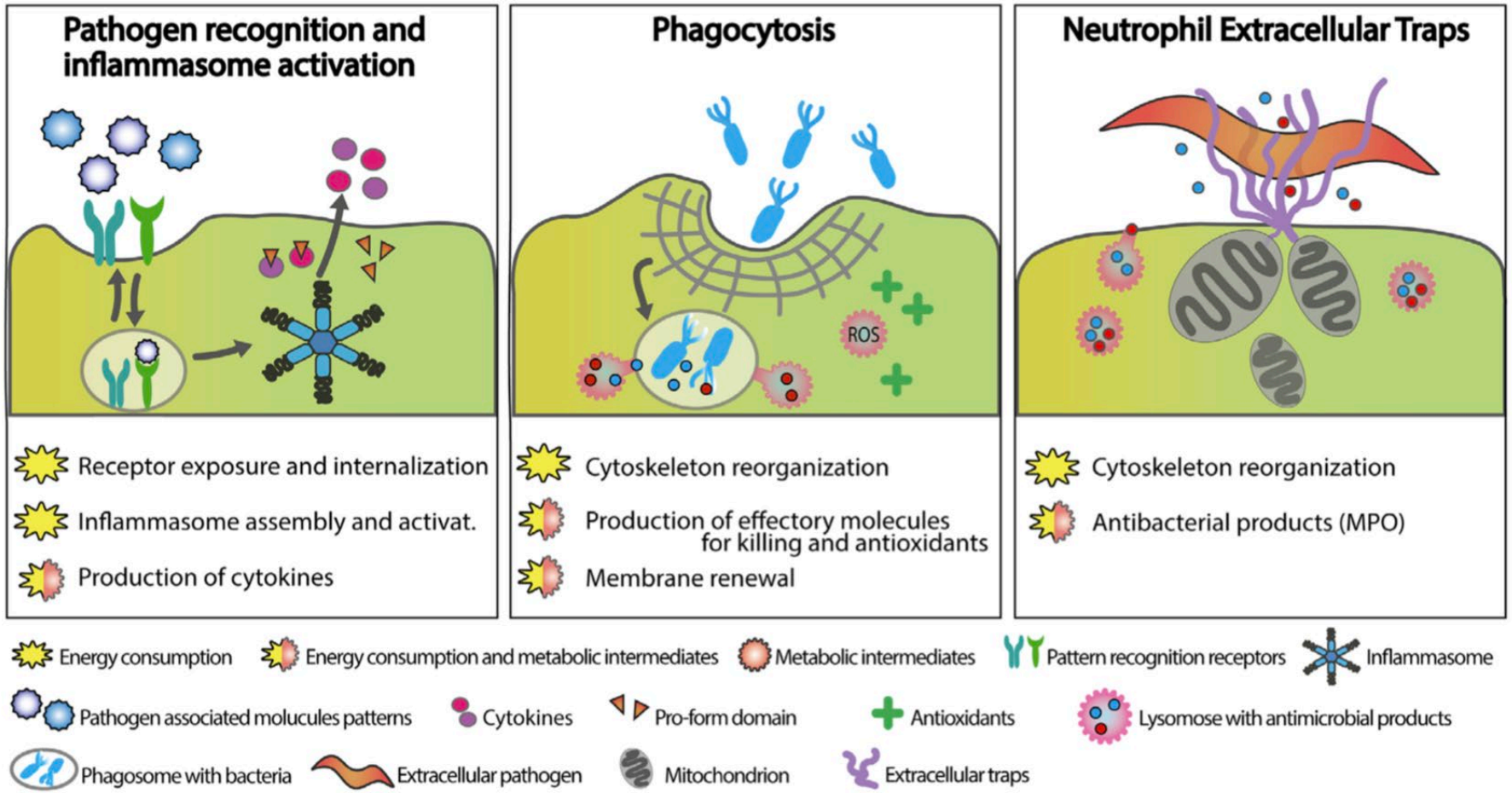
Macrophages

- Hight plasticity from OXPHOS to glycolysis

#immunometabolism

Successful resolution of inflammation depends on nutrient availability supporting the adequate metabolic profiles of immune cells.

Immune cells functions are connected to immunometabolism



The cost of immunity:

- maintaing immune system – 30% basal metabolism
- one cytokine ~ 2300ATP ~ 1150 mol. glucose

Changes in imunometabolism to:

- increase ATP production
- intermedial metabolites production

Key immune processes elicited during inflammation with consideration to energy consumption and the metabolic intermediates