# Research cooperation in autoimmune disorders

**Mechanisms of type 1 diabetes and autoimmune myocarditis pathogenesis**
**Pharmacological modulation in these models**


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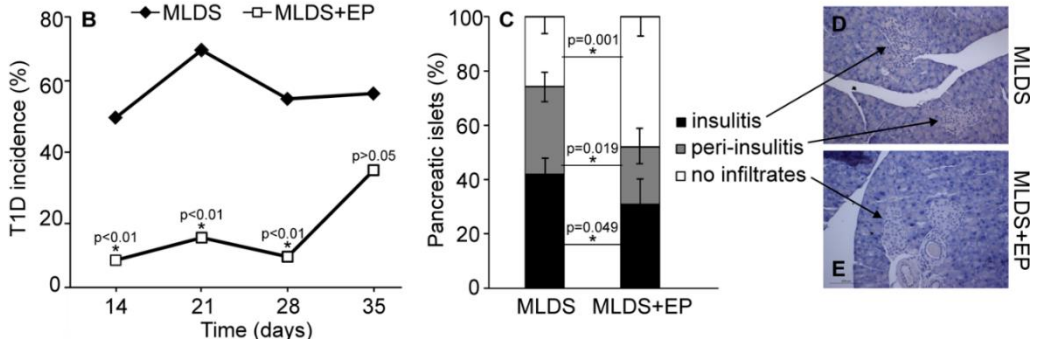
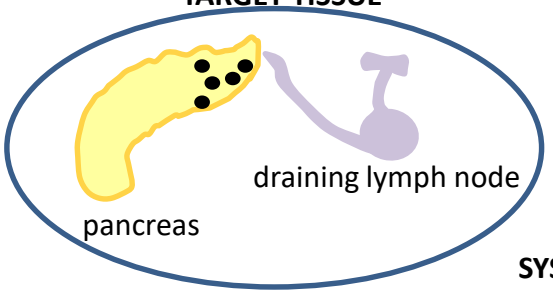
# Model of type 1 diabetes (T1D): Multiple low doses of streptozotocin (STZ)-induced T1D in susceptible C57Bl/6 mice

- Possible routes of drug administration: intraperitoneal, subcutaneous and oral gavage
- Examination of different therapeutic regimes: profilaxis and therapeutic
- Clinical manifestation monitoring: blood glycemia and histological analyses of pancreata




**Ex vivo analyses: delineating mechanisms of action**

**TARGET TISSUE**




**GUT ASSOCIATED LYMPHOID TISSUE**

Peyer's patches
Lamina propria
Intraephitelial lymphocytes

**SYSTEMIC RESPONSE**

Spleen
Peripheral blood

**PHENOTYPE**

Th1
Th2
Th17
Treg
Breg
DC
Mf (M1/M2)
NK
ILCs

**FLOW CYTOMETRY**

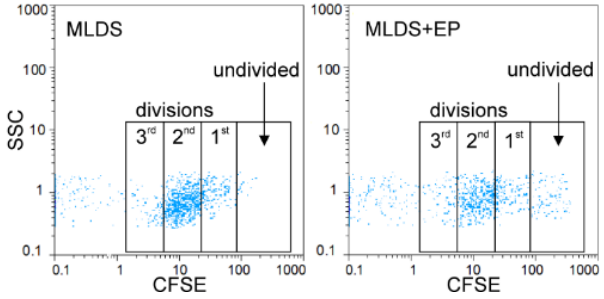
**EXPRESSION/SECRETION OF SIGNATURE CYTOKINES**

IFN-γ
IL-4
IL-17
TGF-β
IL-10
IL-12
TNF
IL-1β

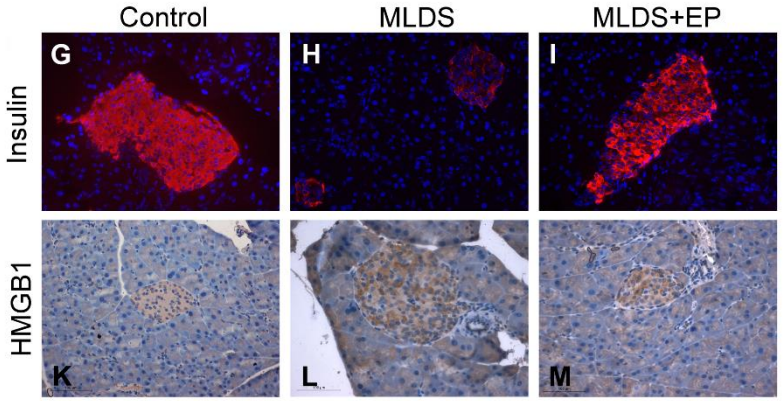
**Real Time PCR/ELISA**

# Comprehensive analyses with the following techniques

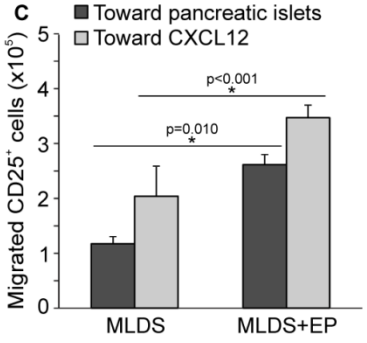
## Proliferation assays: $H^3$-thymidine, CFSE, Ki67, BrdU



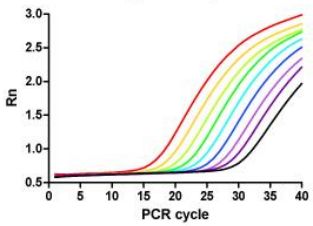

## Immunofluorescent staining
## Immunohistochemistry



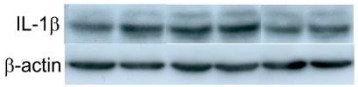

## T cell migration assay



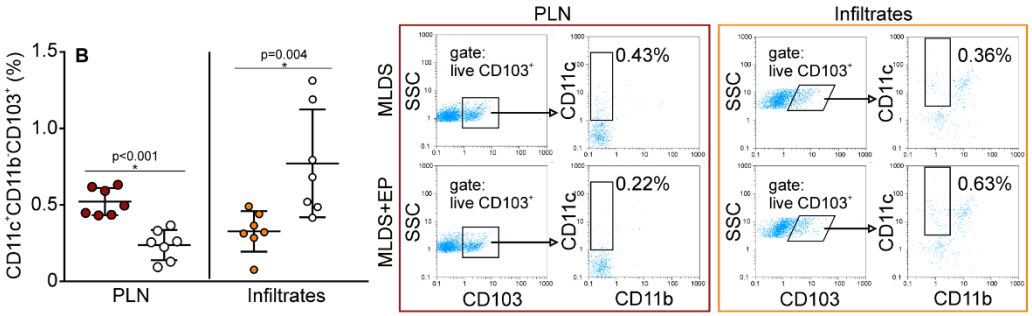

## Real Time PCR



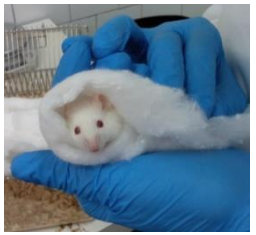

## Western blotting



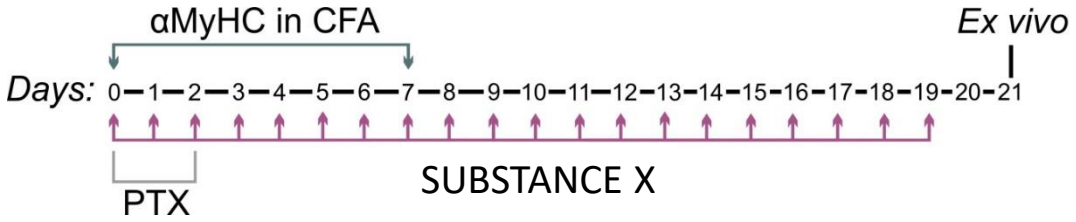

## Flow cytometry



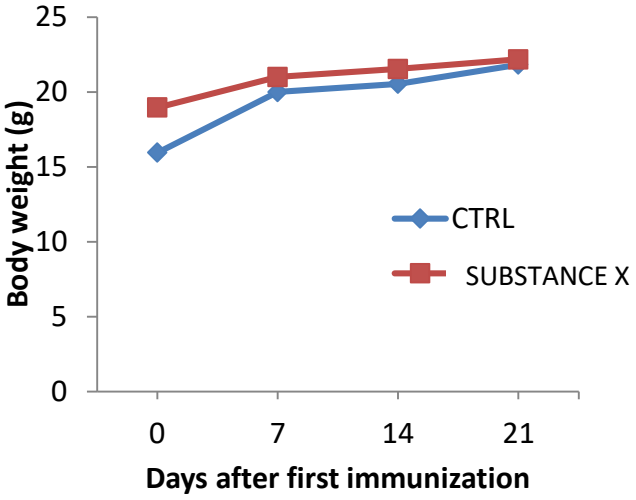
# Model of autoimmune myocarditis: in susceptible Balb/c mice

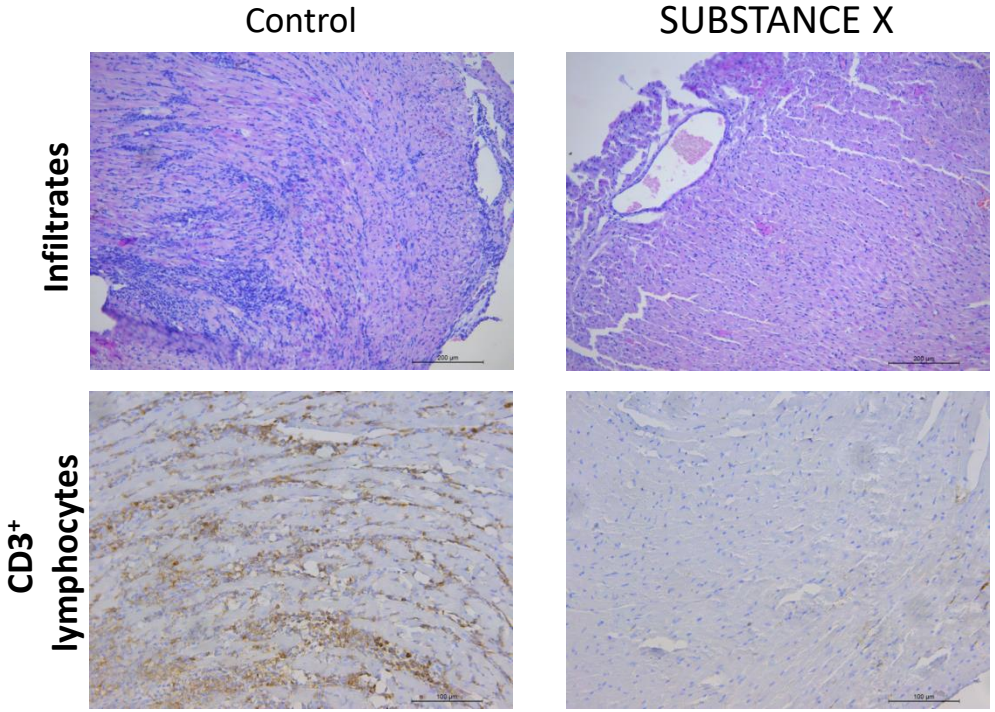


Scheme of disease induction




## Abrogation of the disease by substance x treatment

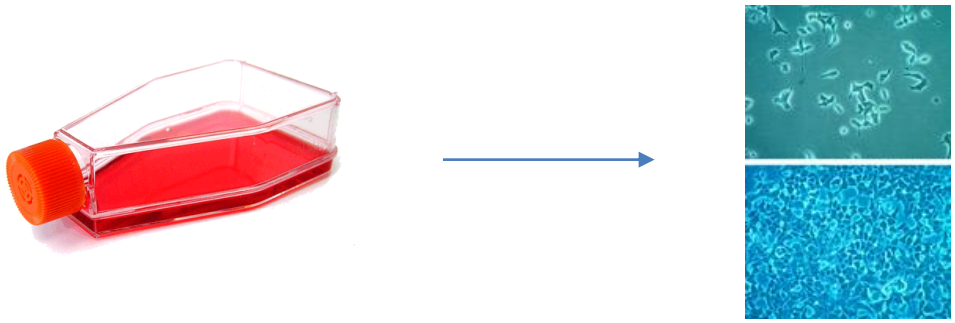



**All previously mentioned analyses are performed also in this model. Target organ: heart.**

# *In vitro* experiments complementing research performed in disease models

ALL SUBSTANCES APPLIED *IN VIVO* ARE ALSO TESTED IN APPROPRIATE *IN VITRO* SETTING TO STRENGHTEN AND ADD DATA ON MOLECULAR MECHANISM



*In vitro* research is performed on:

- Murine pancreatic islets and insulinoma cell lines
- Purified naive T cells instructed toward different Th subtypes (Th1, Th2, Th17, Treg) and purified macrophages and dendritic cells
- Bone marrow-derived dendritic cells

## Relevant references


- Koprivica et al. **Ethyl Pyruvate Stimulates Regulatory T Cells and Ameliorates Type 1 Diabetes Development in Mice.** Frontiers Immunology, 2019.
- Vujicic et al. **Protective effects of carbonyl iron against multiple low-dose streptozotocin-induced diabetes in rodents.** Journal of Cellular Physiology, 2018.
- Nikolic et al. **Standardized bovine colostrum derivative impedes development of type 1 diabetes in rodents.** Immunobiology, 2017.
- Vujicic et al. **Ethyl Acetate Extract of Origanum vulgare L. ssp. hirtum Prevents Streptozotocin-Induced Diabetes in C57BL/6 Mice.** Journal of Food Science, 2016.
- Saksida et al. **Compound A, a selective glucocorticoid receptor agonist, inhibits immunoinflammatory diabetes, induced by multiple low doses of streptozotocin in mice.** British Journal of Physiology, 2014.
- Nikolic, Saksida et al. **Pharmacological application of carbon monoxide ameliorates islet-directed autoimmunity in mice via anti-inflammatory and anti-apoptotic effects.** Diabetologia, 2014.



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## Future perspectives:

1. Delineating basic mechanisms and establishing potential markers in T1D pathogenesis.

2. Translation of studies to a clinical setting. We have established cooperation with the Endocrinology Division in the University Children's Hospital in Belgrade, so we have access to human samples obtained from subjects with T1D.

3. Testing the immunomodulatory potential of novel compounds or herbal extracts in models of T1D and myocarditis, with special emphasis on Treg cells.


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