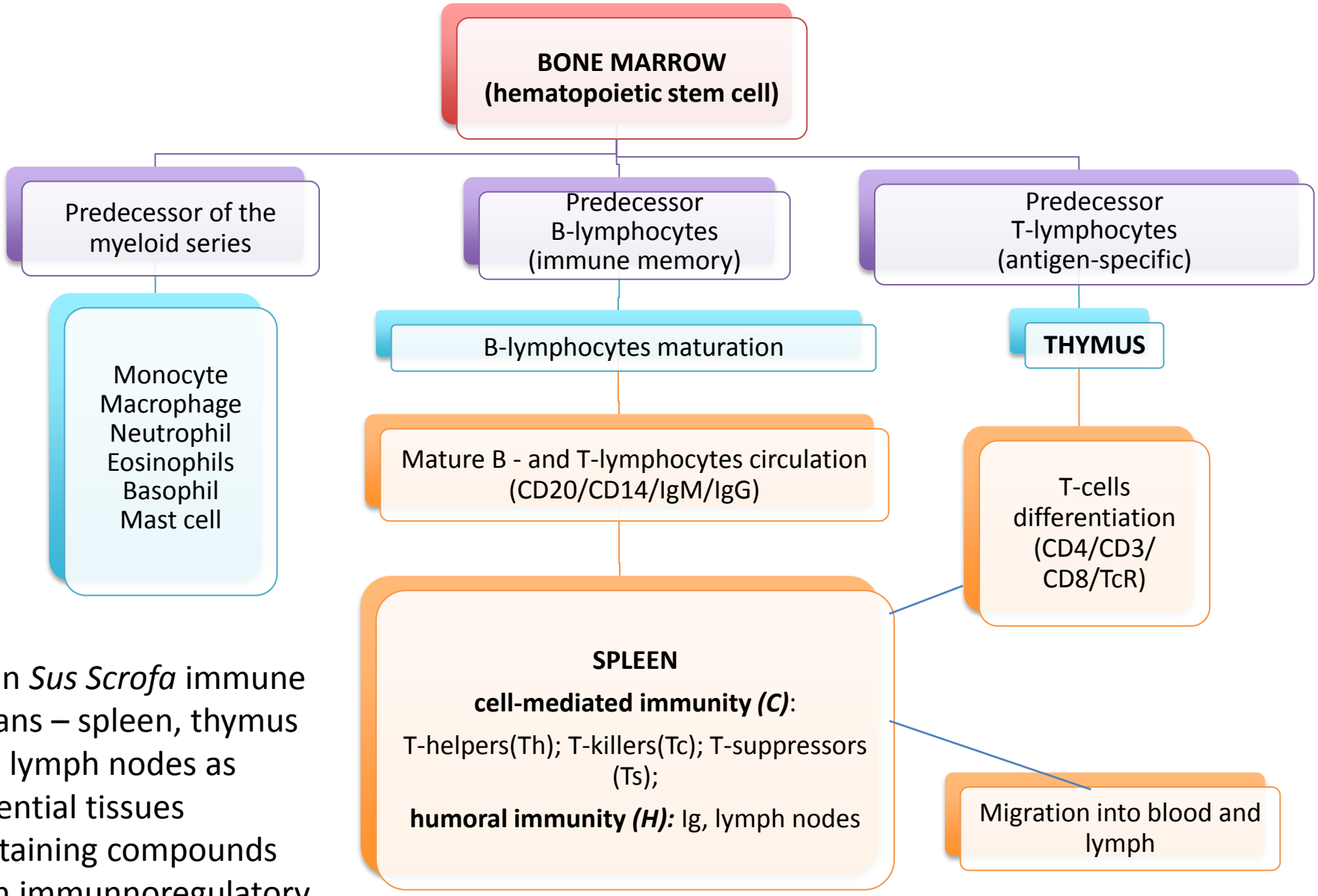
Animal tissue-specific biomolecules influence on rats with cyclophosphamide-induced immunosuppression

Natural immunostimulators obtained over Sus scrofa tissue extraction with the use of water with modified isotope composition (RSF grant No. 15-16-00008)

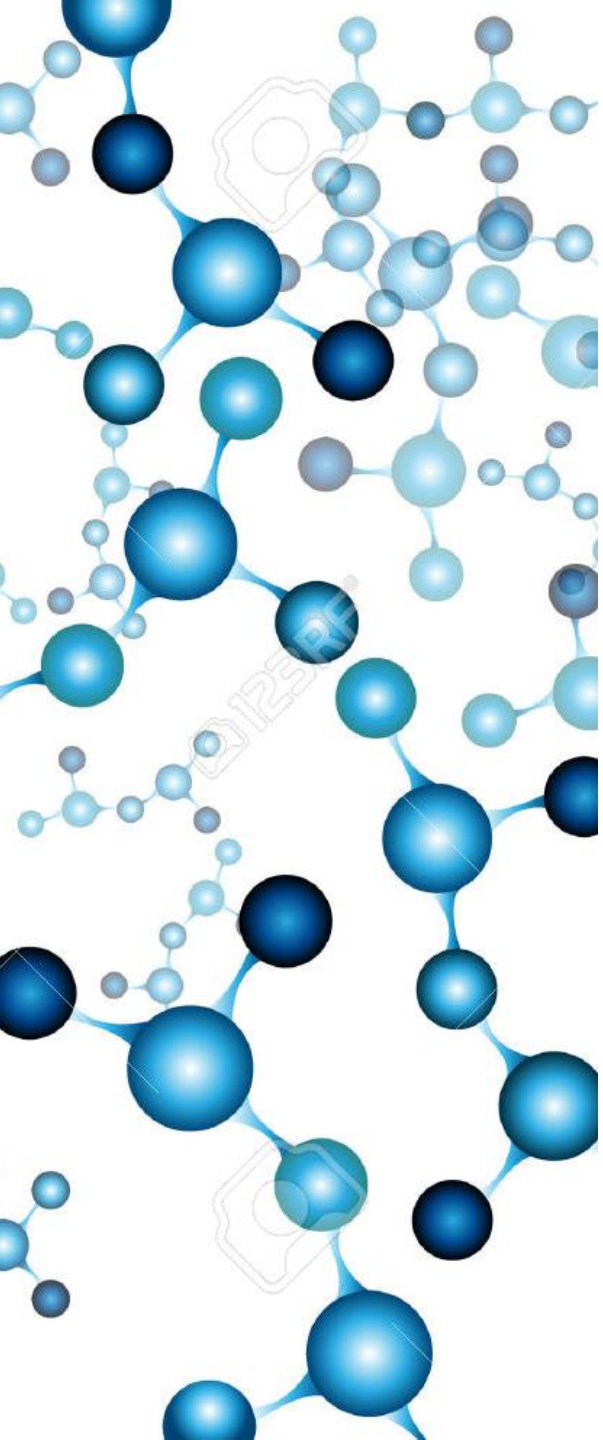
Stepan S. Dzhimak, PhD

Ekaterina R. Vasilevskaya

RAW MATERIALS CHOICE



Main *Sus Scrofa* immune organs – spleen, thymus and lymph nodes as potential tissues containing compounds with immunnoregulatory properties



The aim of the study was to investigate water with modified isotopic D/H composition effect on immunological activity of protein-peptide compounds extracted from *Sus scrofa* immune organs.

Research tasks:

- Research WMIC influence on the protein extractability, peptide and protein profiles;
- Research WMIC complex extracts and its fractions influence in vivo

WMIC – deuterium depleted water with deuterium concentration 40 ppm
DW – standard distilled water with deuterium concentration 140 ppm

EXTRACTION ALGORITHM

Grinding
- Knives with ceramic coating

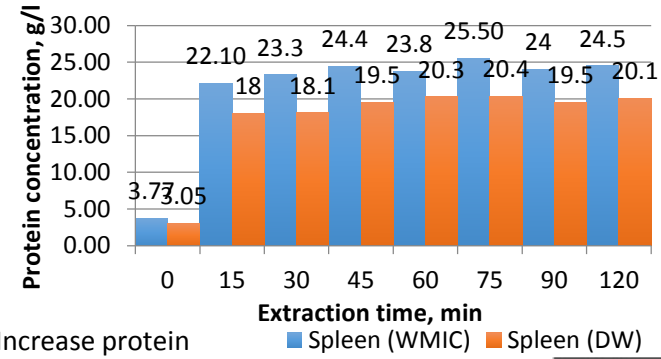
Extraction
- 4°C, 0,9 % solution NaCl - WMIC, 4 hours
- 4°C, 0,9 % solution NaCl - DW, 4 hours

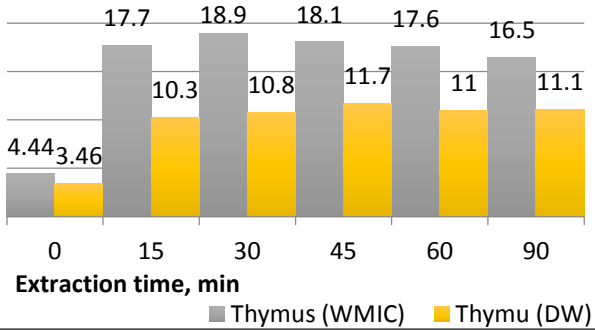
Centrifugation
- 3500 Rev/min, 8 min,
  Plastic tubes

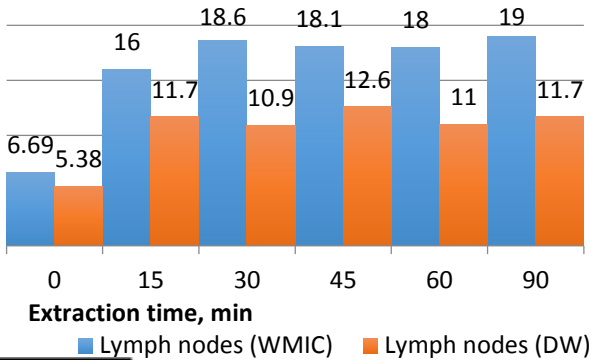
Ultrafiltration
- Pressure 2.5 bar
  Polyethersulfone membranes with plastic fittings and tanks

Lyophilic drying
- Pressure 3,3 Pa, T = (-41±1°C)
- Glassware

WMIC and DW EXTRACTS COMPARATIVE ANALYSIS

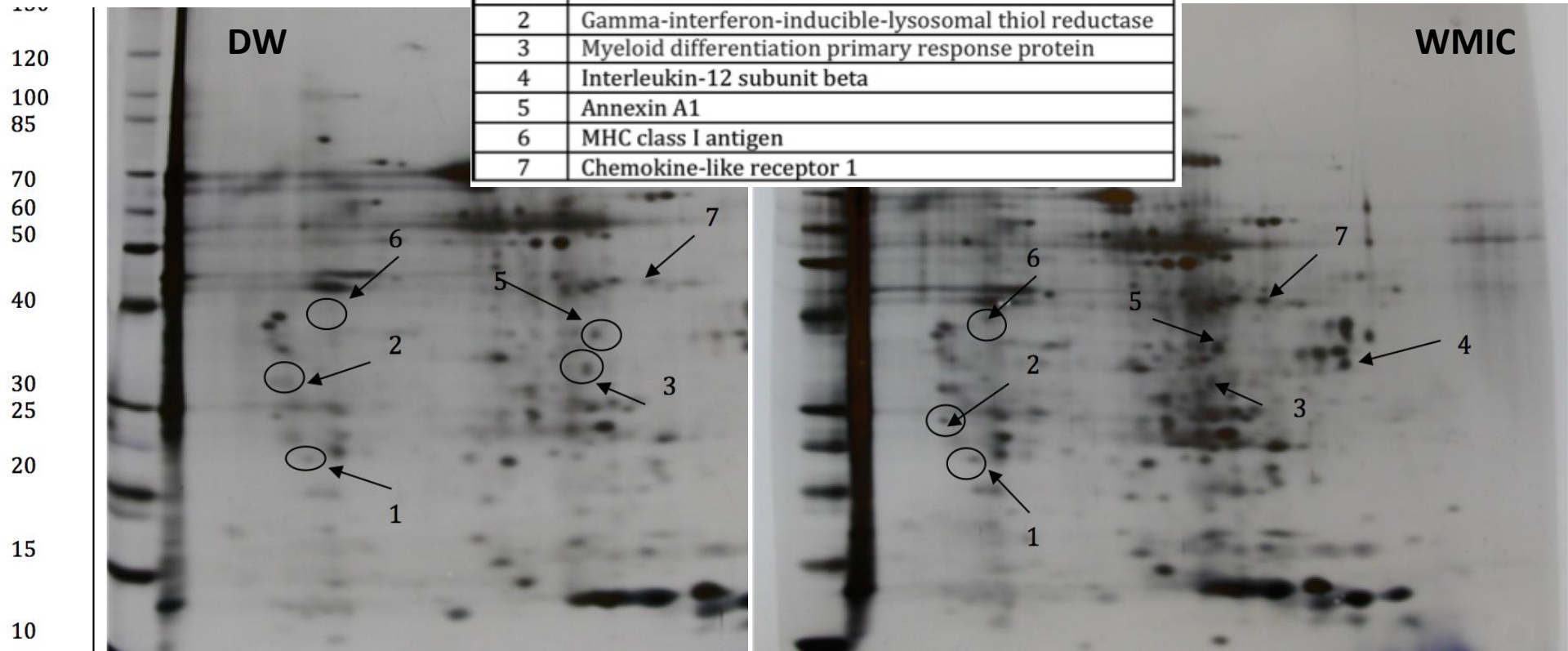






Increase protein concentration from 15 to 50 %

№№	Protein
1	Interferon beta protein
2	Gamma-interferon-inducible-lysosomal thiol reductase
3	Myeloid differentiation primary response protein
4	Interleukin-12 subunit beta
5	Annexin A1
6	MHC class I antigen
7	Chemokine-like receptor 1



IMMUNOLOGICAL REACTIVITY *IN VIVO*

Study of immune corrective effect was carried out with:

Laboratory animals

- **Male Wistar rats, SPF**
- **N= 58, m = 390 ± 10 r**

Immunodeficiency Model

- **Modulator: cyclophosphamide (Sigma)**
- **Intraperitoneal injection**
- **Dose: 75 mg/kg**
- **Interval: 72 hours,**
- **Repeat count: three times**
- **Model complete: 12 days after first injection**

Research (20 days)

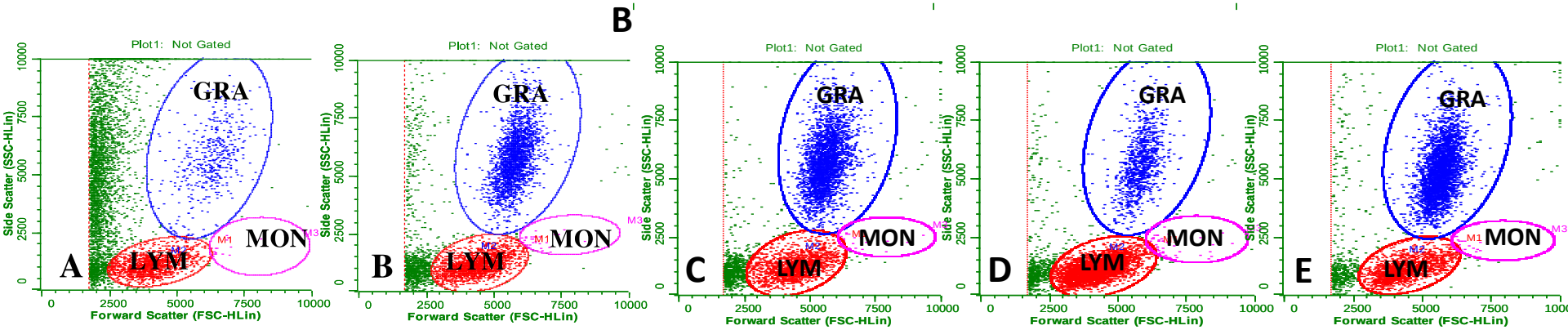
- **Group A (n=10) – intact animals**
- **Group B (n=10) – control animals (IDF model)**
- **Group C (n=10) –20 days treatment, WMIC fraction up to 5 kDa**
- **Group D (n=10) – 20 days treatment, WMIC fraction 5-30 kDa**
- **Group E (n=10) – 20 days treatment, WMIC fraction above 30 kDa**

Методы

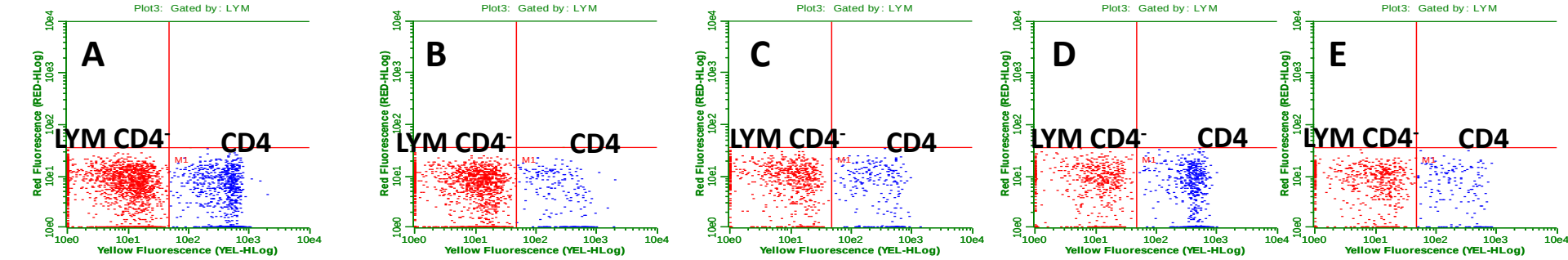
- **Cytometry analysis : LYM, MON, GRA; CD4.**
- **Immunoassay analysis:**
- **Complement components C1q,C1qA, C1qB, C3,C4, C5**
- **IgM, IgG, Il-2, Il-4, Il-6**

IMMUNOPHENOTYPING

Lymphocytes, monocytes, granulocytes content.



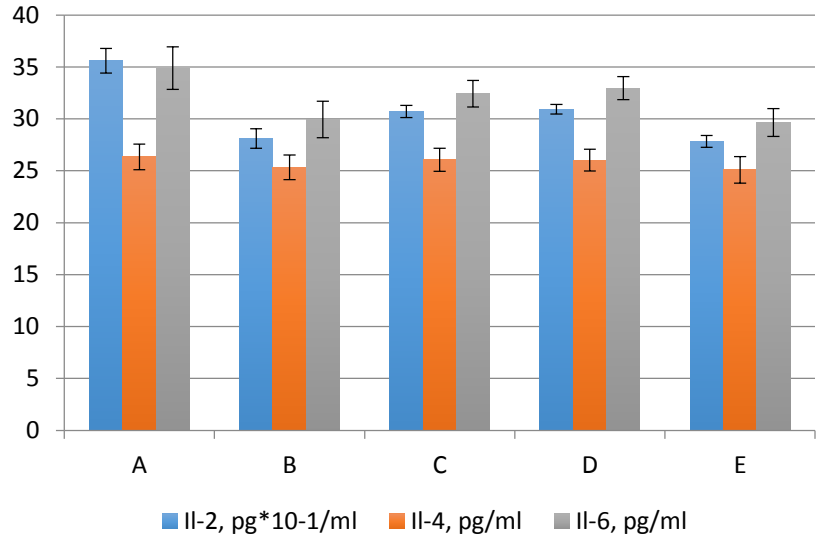
CD4 content

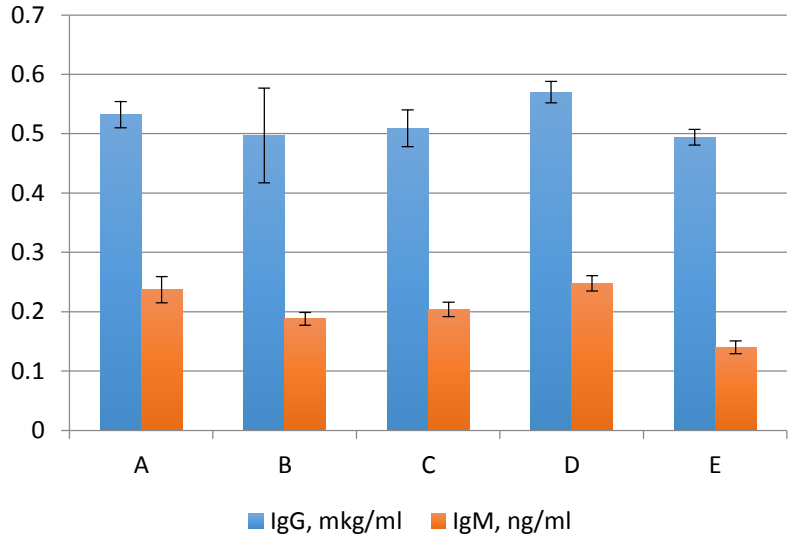


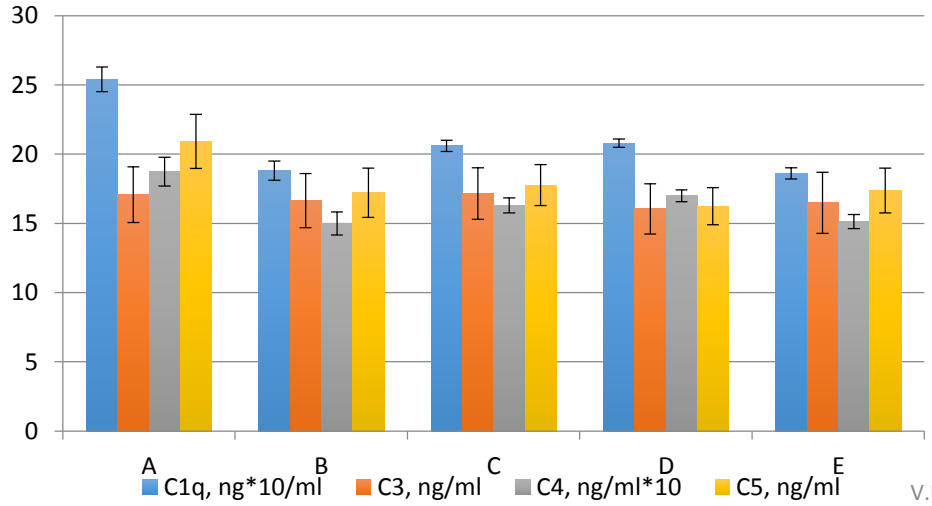
A – intact; B – control; C – fraction <5 kDa;
D – fraction 5-30 kDa, E –fraction >30 kDa.

IMMUNOASSAY ANALYSIS RESULT

A – intact; **B** – control; **C** – fraction <5 kDa;
D – fraction 5-30 kDa, **E** –fraction >30 kDa







CONCLUSION

- Deuterium depleted water intake led to the increase proteins (involved in the immune response) concentration during extraction from animal tissues (spleen, thymus, lymph nodes);
- *In vivo* research showed immune system recovery, adaptive immune response and functional activation of nonspecific immune defense system;
- Biomolecules (5-30 kDa), isolated from *Sus Scrofa* immune tissues by WMIC extraction, showed obvious immunoactivating effect.

THANK YOU!

Stepan S. Dzhimak
jimack@mail.ru

.

This work was supported by Russian Science Foundation (RSF) grant No. 15-16-00008.
"Development of innovative natural adaptogenic stimulants of innate (nonspecific) immunity based on species and tissue-specific biomolecules"

РНФ | Российский научный фонд

ВНИИМП им. В.М. Горбатова