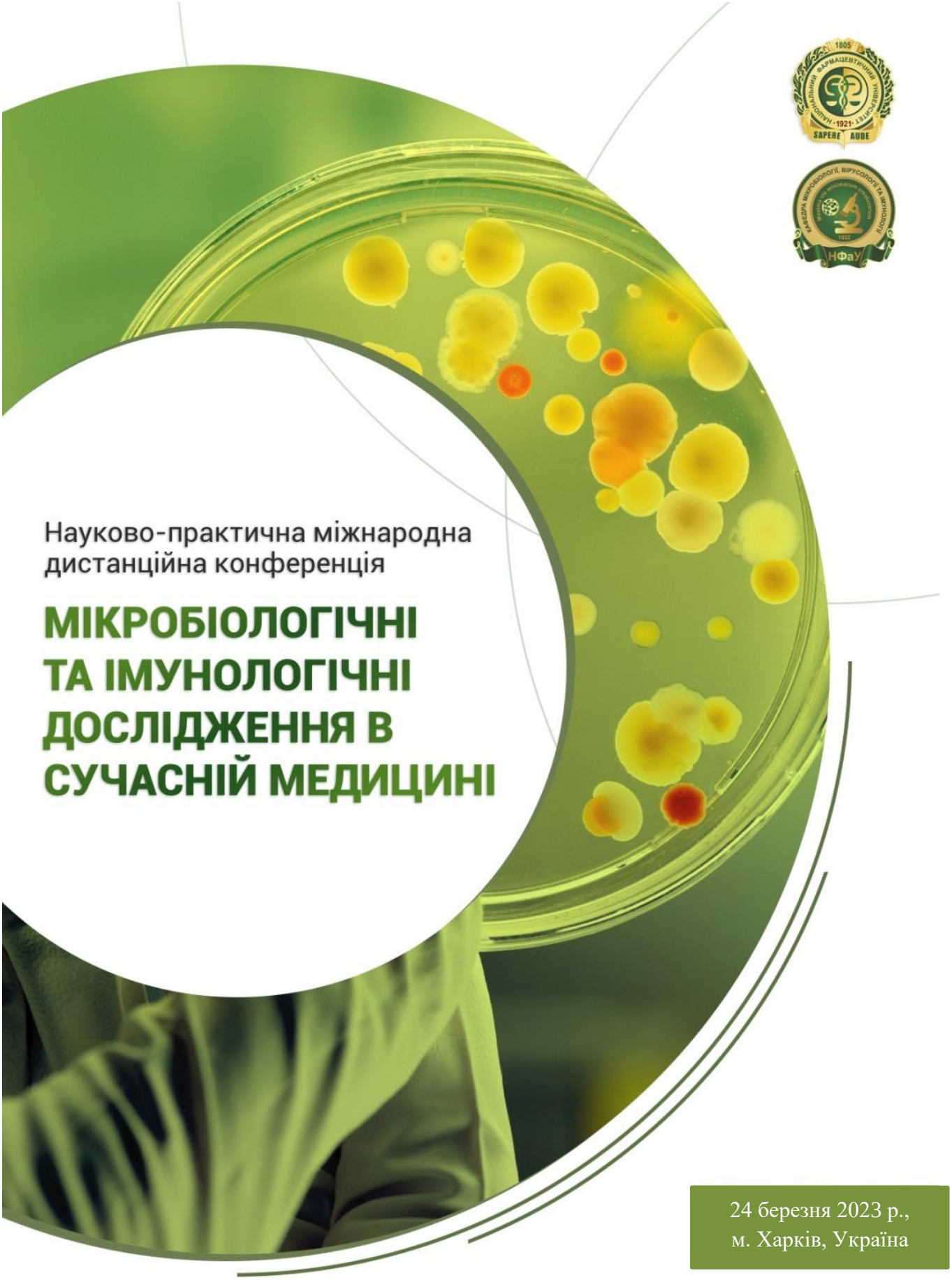

Науково-практична міжнародна
дистанційна конференція

МІКРОБІОЛОГІЧНІ ТА ІМУНОЛОГІЧНІ ДОСЛІДЖЕННЯ В СУЧАСНІЙ МЕДИЦИНІ

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НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ
КАФЕДРА МІКРОБІОЛОГІЇ, ВІРУСОЛОГІЇ ТА ІМУНОЛОГІЇ

MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
DEPARTMENT OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

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– the relevant Standard Operating Procedures of the laboratory should describe procedures aimed at preventing the risk of developing laboratory infections among medical personnel.

Conclusions. Prevention of the spread of dangerous biological agents is possible with strict adherence to standard rules of work in medical laboratories and manipulation techniques in combination with the use of primary (safe equipment) and secondary barriers (special laboratory design).

USE OF THE SECRETOME OF MESENCHYMAL STEM CELLS IN PHARMACEUTICAL BIOTECHNOLOGY

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The modern pharmaceutical industry is based more on advances in biotechnology than in chemical synthesis. Many processes, from the microbial synthesis of antibiotics, vitamins and recombinant proteins to the production of monoclonal antibodies and regenerative medicine, are associated with the cultivation of cells and the use of these cells or their metabolic products as therapeutic agents.

The last decades using of living cells of both prokaryotes and eukaryotes in treatment has become increasingly common. So, in particular, a team of researchers led by Rocco Mazzolini created a transgenic strain of *Mycoplasma pneumoniae* for the treatment of pneumonia caused by *Pseudomonas aeruginosa*. The administration of a living microorganism makes it possible to overcome the protective biofilm of a pathogenic bacterium and kill it with an antimicrobial agent.

Mesenchymal stem cells (MSCs) – species or individual – are the most frequent objects of pharmaceutical biotechnology among eukaryotic cells. Drugs based on these cells are being developed. Morgan T. Sutton et al. showed that MSCs are able to produce *in vitro* substances that exhibit antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. However, mesenchymal stem cells are rarely used as antimicrobial agents. They have four important properties that find applications in regenerative medicine: MSCs stimulate tissue regeneration, reduce inflammation, scarring, and modulate the immune system.

The use of living cells carries a number of risks associated with their potential transformation. Bacterial cells can acquire pathogenic properties, and

mesenchymal stem cells can undergo neoplastic transformation, atypical differentiation, or be eliminated from the body before reaching a therapeutic effect. The described difficulties force the doctor to weigh the risks and benefits of using cell therapy in each individual clinical case.

And if the use of therapeutic strains of bacteria (such as described *Mycoplasma pneumoniae*) is planned in exceptional cases (with the development of superinfection), then regenerative medicine can be widely used. This circumstance makes researchers pay attention to the substances produced by mesenchymal stem cells and called secretome. The secretome of MSCs consists of soluble components (mainly peptides) and vesicles containing various biologically active substances, mainly microRNAs. Researchers are studying the individual components of the MSC secretome and their contribution to the described four main properties, however, the separation of MSCs exometabolites into individual components that are similar in solubility and mass will be difficult and expensive if used in the pharmaceutical industry.

In view of the above, it seems interesting to study the pharmacological properties and toxicity of the secretome of mesenchymal stem cells obtained under various conditions, as well as its individual fractions, which are obtained by separation using relatively simple and inexpensive methods.

The safety and efficacy of the whole secretome fraction of mesenchymal stem cells cultured under standard conditions (37°C, 5% CO_2) without the addition of any differentiation or secretion inducers was studied. The secretome was collected from a monolayer culture of the 3rd passage, containing 500-600 thousand cells per ml at the logarithmic stage of growth, and purified from cell debris using centrifugation (3000 rpm, 10 min.). Standardization of the secretome of different batches was carried out according to two parameters: protein concentration (at least 2.5 mg/ml) and mitogenic activity in relation to the fibroblast culture when added at a concentration of 10% to the culture medium (exceeding the control by at least 10%).

The toxicological study included the study of acute toxicity in mice with intramuscular (i/m) and subcutaneous (s/c) administration of samples. Pharmacological properties of MSCs secretome were evaluated by enhancing antibody production and skin regeneration. The effect on immune function was researched in mice by determining the number of antibody-forming cells (AFC) in the spleen according to the method of Ierne K.N. et al. and studying the titer of hemagglutinins (TH) after immunization with ram erythrocytes against the background of i/m injection of the whole secretome of MSCs at doses of 10-50 μl/kg (control – saline). The regenerative effect of the secretome of MSCs was studied in the model of stencil wounds in rats.

During the study of acute toxicity of secretome samples, it was found that a single injection of the sample at doses of 25 ml/kg i/m and 50 ml/kg s/c did

not cause intoxication and death of mice of both sexes, which was confirmed by observation of animals, determination of body weight dynamics, and macroscopic examination internal organs at autopsy (without visible pathologies) performed on the 14th day after the administration of the sample.

A statistically significant and dose-dependent increase in AFC by 3-10.5 times, and the TH in the blood by 1.2-2.0 times was shown after administration of samples of the secretome. It was shown that by the 11th day of observation, s/m injection of the sample at doses of 10 and 50 µl/kg significantly reduced the area of the wound surface compared to the control. The healing rate at the two studied doses was 77% for 10 µl/kg and 72% for 50 µl/kg (differences between doses are not statistically significant).

Thus, the absence of acute toxicity and the presence of immunostimulatory and regenerative properties in the MSC secretome obtained by the described method were shown. This method can be used to obtain a basic pharmaceutical substance from which regenerative medicine can be obtained.

Arin B. Aurora et al. note the contribution of the immune system to skin regeneration and describe the following probable mechanism, which we partially observed in the study: early and late macrophages (similar to classically activated, M1, or alternatively activated, M2) are secreting TGFβ, EGF, VEGF, IGF, that leads to activation of fibroblasts and endothelial cells proliferation; this ensures the replacement of damaged cells, from the debris of which the zone has been cleared by macrophages, fibroblasts, keratinocytes.

Further studies can be aimed at studying the properties of individual fractions of the basic secretome obtained by separating the components by mass by ultrafiltration, as well as the modified secretome using physical, chemical or biological inducers of differentiation or secretion.

SALMONELLA PULLORUM EFFECTOR STEE REGULATES TH1/TH2 BALANCE BY TRIGGERING THE STAT3/SOCS3 AXIS THAT SUPPRESSES NF-κB ACTIVATION

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Abstract: *Salmonella enterica* serovar Pullorum (*S*. Pullorum) can escape the clearance of the host immune system by some special effector proteins to enhance its intracellular survival and growth. Our previous study showed that *steE*, an anti-inflammatory effector protein, is closely associated with enhanced the persistent infection of *S*. Pullorum by regulating host inflammatory response. However, the mechanism via which *steE* regulates Th1/Th2 balance remains unclear in the case of *S*. Pullorum infection. In this