Research project objectives/Research hypothesis

Evolutionarily conserved proteins play a fundamental role in maintaining physiological link between classes and phyla of the animal kingdom. They are low-specific but endowed with bewildering spectrum of functions by acting on numerous physiological processes.
To date, there exist gaps in the knowledge regarding the role of some eggs' proteins in the embryo development of birds. The lack of a direct association between the maternal and embryo, in contrast to mammals, suggests that an egg should contain all the bioactive constituents for its developing. The role of certain proteins, such as ovotransferrin, vitellogenin, phosvitin and immunoglobulin Y in birds' embryo development and protection has been clarified, but limited data is available about other proteins, such as yolkin – a product of vitellogenin degradation. Based on the hitherto obtained results we assume that, in egg-laying animals, yolkin may fulfill an analogous role like immunotropic proteins and peptides (such as colostrinin and lactoferrin) in the colostrum/milk of mammals in immune system development and function. The aim of the project is to establish the immunoregulatory and immunorestorative properties of yolkin, as well as to determine the cellular and molecular mechanism of its action. Due to a highly conservative structure of the vitellogenin within vertebrates, the project may be performed in generally approved and routine used in research mouse experimental models. To achieve the research objective, we intend to use experimental *in vivo* models to establish if yolkin will: promote maturation and differentation of the immune cells, accelerate the immune function in suppression after chemotherapy, ameliorate inhibitory psychic stress impact on the immune response, restrain inflammation and bacterial infection and prevent abortion through correction of the immune status during pregnancy. The molecular and cellular mechanisms of yolkin action will be confirmed by *in vitro/ex vivo* models for analysis of: antiviral effect, cytokine production, cell phenotypes and signaling pathways in cultured immune cells isolated from mouse lymphoid organs (bone marrow, thymus, spleen and lymph nodes) and cell lines. Results of this project enable conclusions to be drawn regarding the role of yolkin in the development of birds' embryos.

Research project methodology

1. Preparation of yolkin from yolk of hens' eggs, lyophilization and confirmation of homogeneity of the received preparation; pool from several batches of isolated yolkin (as polypeptide complex), derived from separate purification procedures will be prepared for use in the whole project.
2. *In vitro* tests (on cells incubated with yolkin in cultures): •cytokine production in thymocytes, splenocytes and lymph node cells stimulated with bacterial lipopolisaccharide; •expression of signaling proteins in mouse resident immune cells and immune cell lines representing macrophages and natural killer cells; •level of cyclooxygenase-2 (COX-2) in the macrophage cell line; •antiviral effect in hen fibroblast cell line.
3. *In vivo* tests: •humoral and cellular immune response tests in weanling mice to study the ability of yolkin to acceleration of maturity of immature immune system; •cellular immune response test in mice in long-term psychic (immobilization) stress to study the ability of yolkin to restore stress-impaired immune system; •humoral and cellular immune response tests in mice treated with a sublethal dose of cyclophosphamide to study the ability of yolkin to restore chemotherapy-impaired immune system; •lipopolysaccharide-induced inflammation to evaluate protective effect of yolkin in inflammation; •bacteremia after administration of *Escherichia coli* to study anti-infectious activity of yolkin; •abortion in pregnancy abortion prone mice CBA/J×DBA/2J to study the effect of yolkin on correction of the immune status during pregnancy
4. *Ex vivo* tests (on cells isolated from mice treated with yolkin): •cytokine and nitric oxide production in cells from lymphoid organs, stimulated with lipopolisaccharide; •cell phenotype of immune cells (T and B cells, macrophages, dendritic cells) isolated from lymphoid organs; •histological study of tissue.
In *in vivo* models yolkin will be administered to mice orally (in drinking water) or repeatedly intraperitoneally.

Expected impact of the research project on the development of science

The results will reveal a possible role of yolkin in the development of the birds' embryonic immune system. Eggs' proteins are evolutionarily old with a similar structure and role in all egg-laying animals from insects to birds. Therefore, we assume that our results should fill the existing knowledge gaps regarding the nature of proteins responsible for immune function in egg-laying animals. The results, if they confirm immunoregulatory properties of yolkin, may also initiate subsequent steps of preclinical and clinical trials aimed at application of yolkin as a nutraceuticals/diet supplements in prevention/treatment of various immunological disorders, immune deficiencies and infections in human medicine and veterinary.