## Project summary

The central goal of the research was formulated based on data indicating that: i) the world’s aged population grows progressively; ii) at leаst 80% of aged individuals is afflicted with at least one chronic disease as a result of changes in immune function, and iii) longer life-span in female subjects reflects sex bias in age-associated immune system decline (phenomenon known as immunosenescence), which, at least partly, can be linked with sex differences in circulating levels of gonadal steroids. The CENTRAL GOAL of the research was to investigate: 1) influence of AGING on immunological reactivity of female rodents through analysis of (i) PHENOTYPIC AND FUNCTIONAL PROPERTIES OF INNATE AND ADAPTIVE IMMUNЕ CELLS; (ii) DEVELOPMENT OF AUTOIMMUNE DISEASES and (iii) EFFICACY OF PROTECTIVE IMMUNE RESPONSE and 2) significance of changes in circulating levels of gonadal steroids (estrogens) for the age-associated immune alterations. We planned to achieve this goal through THREE SPECIFIC SUBAIMS, using mainly rat models as (i) data on effects of aging on rat immune system are limited and inconsistent and (ii) rat immune system exhibits some striking analogies with that in humans (e.g. in some characteristics of activated T cells, and phenotypic and functional properties of some subsets of dendritic cells). In SPECIFIC SUBAIM 1, we aimed to: 1) investigate influence of aging on (i) phenotypic and functional (e.g. production of inflammatory mediators) characteristics of MACROPHAGES (МФ); (ii) capacity of DENDRITIC CELLS (DC) to present antigens to CD4+ T cells, activate them and direct the development of distinct T helper (Th) cell subsets, and (iii) CD4+/CD8+ T-cell ratio (immunoregulatory index) in various immune compartments and phenotypic properties of their T CELLS, mainly CD4+ ones, such as expression of regulatory cell markers and ratio of cell subsets at distinct stages of differentiation/maturation (recent thymic emigrants, naïve and activated/memory cells), and contribution of the putative alterations in thymopoietic efficiency and the synthesis of T-cell homeostatic cytokines in the periphery (as they are of crucial importance for the peripheral T-cell pool maintenance) to the putative changes in the characteristics of the peripheral T-cells, and 2) explore significance of changes in circulating levels of gonadal steroids for the putative effects of aging on the cells of innate and adaptive immune system. Given that data on influence of aging on development of autoimmune diseases in rodents are limited and inconsistent, in SPECIFIC SUBAIM 2 we planned to: 1) examine influence of aging on induction and severity of organ-specific autoimmune diseases using experimental models characterized by dominant pathogenetic role of CD4+ T cells–models of multiple sclerosis (EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS, EAE) and RHEUMATOID ARTHRITIS (collagen-induced arthritis, CIA), the diseases typically exhibiting clinical onset in distinct adult age, and mechanisms underlying putative age-related alterations in their incidence and clinical presentation, and 2) investigate relevance of alterations in circulating levels of gonadal steroids for the putative changes in inducibility and clinical presentation of EAE and CIA. In SPECIFIC SUBAIM 3, we planned to explore: 1) influence of aging on CD4+ T-cell-dependent PROTECTIVE IMMUNE RESPONSE using a rodent influenza-vaccinationmodel through analysis of parameters of (i) efficacy of follicular CD4+ T-cell help to B cells and (ii) humoral immune response (e.g. serum titers of IgG antibodies, their affinities and subclass profiles, and serum titers of functional antibodies), and 2) significance of alterations in circulating levels of gonadal steroids for its effects. RELEVANCE: To advance our understanding of immunosenescence phenomenon and thereby form the scientific base for designing new approaches in moderating/preventing deleterious effects of aging.

Keywords: aging, sex differences, immune response, experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA), protective immune response

## Sažetak projekta OI 175050

Centralni cilj istražavanja je postavljen imajući u vidu da: a) se udeo starih u opštoj populaciji progresivno povećava, b) najmanje 80% populacije starih boluje makar od jedne bolesti u čijoj patogenezi dominantnu ulogu imaju starenjem uslovljene promene u imunskom sistemu i v) duži očekivani životni vek kod žena umnogome odražava polne razlike u starenju imunskog sistema (tzv. imunološkom starenju), što se delimično povezuje sa polnim razlikama u koncentraciji gonadnih steroida u cirkulaciji. CENTRALNI CILj ISTRAŽIVANjA bio je da se ispita: 1) uticaj STARENjA na imunološku reaktivnost životinja ženskog pola analizom (a) FENOTIPSKIH I FUNKCIJSKIH KARAKTERISTIKA ĆELIJA UROĐENE I STEČENE IMUNOSTI, (b) RAZVOJA AUTOIMUNSKIH BOLESTI i (v) EFIKASNOSTI PROTEKTIVNOG IMUNSKOG ODGOVORA i 2) značaj promena u koncentraciji gonadnih steroida (estrogena) u cirkulaciji za promene u imunskom sistemu tokom starenja. Planirano je da se centralni cilj realizuje kroz TRI SPECIFIČNA POTCILjA, a da se istraživanja većinski obave na pacovima, budući da su podaci o uticaju starenja na imunski sistem pacova, za razliku od njegovog uticaja na imunski sistem miša, oskudni i da je, makar u nekim imunskim parametrima, npr. karakteristike aktivisanih T-limfocita, fenotip i funkcija nekih subpopulacija dendritskih ćelija (DĆ), pacov, u poređenju sa mišem, „sličniji“ čoveku. PRVI SPECIFIČNI POTCILj bio je da se: 1) ispita uticaj starenja na (a) fenotipske i funkcijske (sekrecija proinflamatornih medijatora) karakteristike MAKROFAGA (MΦ), (b) sposobnost DĆ da prezentuju antigene CD4+ T-limfocitima, da ih aktivišu i usmere njihovu diferencijaciju ka određenim subpopulacijama T-pomoćničkih (Th) ćelija i (v) odnos CD4+ i CD8+ T-LIMFOCITA (tzv. imunoregulatorni indeks), fenotipski profil T-limfocita, prevashodno CD4+ T-ćelija (kao ključnih za stečenu imunost), u smislu zastupljenosti ćelija na različitim stadijumima diferencijacije/sazrevanja (sveži timusni emigranti, naivne i aktivisane/memorijske ćelije) i ćelija regulatornog fenotipa, u različitim imunskim odeljcima (periferna krv, sekundarni limfni organi), kao i doprinos promena u efikasnosti timopoeze i delovanju homeostatskih citokina na periferiji (kao ključnih mehanizama u održavanju homeostaze perifernog T-ćelijskog imunskog odeljka ) promenama T-limfocita na periferiji tokom starenja i 2) istraži značaj promena u koncentraciji gonadnih steroida u cirkulaciji za efekte starenja na ćelije urođene i stečene imunosti. Imajući u vidu da su podaci vezani za uticaj starenja na razvoj autoimunskih bolesti kod eksperimentalnih životinja oskudni i, u velikoj meri, kontradiktorni, DRUGI SPECIFIČNI POTCILj bio je da se: 1) ispita uticaj starenja na inducibilnost i težinu organ-specifičnih autoimunskih bolesti korišćenjem eksperimentalnih modela u kojima CD4+ T-limfociti imaju dominantnu patogenetsku ulogu–modela multiple skleroze (EKSPERIMENTALNI AUTOIMUNSKI ENCEFALOMIJELITIS, EAE) i reumatoidnog artritisa (KOLAGENOM INDUKOVANI ARTRITIS, KIA), kao bolesti koje se predominantno javljaju u mlađem (EAE) i starijem (KIA) adultnom uzrastu, i definišu ćelijski i molekularni mehanizmi odgovorni za, moguće, starenjem uslovljene promene u kliničkoj prezentaciji ovih bolesti i 2) istraži značaj promena u delovanju gonadnih steroida za ove promene. TREĆI SPECIFIČNI POTCILj ISTRAŽIVANjA bio je da se: 1) ispita uticaj starenja na PROTEKTIVNI IMUNSKI ODGOVOR zavisan od CD4+ T-limfocita na modelu vakcinacije sezonskom vakcinom protiv gripa, analizom parametara (a) efikasnosti folikulskih CD4+ T-limfocita da pruže pomoć B-limfocitima i (b) humoralnog imunskog odgovora (npr. titar i afinitet IgG antitela, profil IgG potklasa, titar funkcionalnih antitela) i 2) istraži značaj promena u delovanju gonadnih steroida za efekte starenja. ZNAČAJ ISTRAŽIVANjA: Da se razumevanjem starenjem uslovljenih promena u imunskom sistemu stvori naučna baza za dalja ispitivanja mogućnosti da se neželjeni efekti starenja ublaže/spreče.

Ključne reči: starenje, polne razlike, imunski odgovor, eksperimentalni autoimunski encefalomijelitis (EAE), kolagenom-izazvan reumatoidni artritis (KIA), protektivni imunski odgovor

## Selected results/Odabrani rezultati

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