ILG‘OR TEXNOLOGIYALAR MARKAZI HUZURIDAGI DSC.03/30.07.2024.B.179.01 ILMIY DARAJALAR BERUVCHI ILMIY KENGASH

IlG’OR TEXNOLOGIYALAR MARKAZI

MUMINOV MUZAFFAR ISLOMJONOVICHNING

SARS-COV-2 VIRUSIGA QARSHI REKOMBINANT OQSIL VAKSINA YARATISH

03.00.03 –Molekulyar biologiya. Molekulyar genetika. Molekulyar biotexnologiya

BIOLOGIYA FANLARI BO‘YICHA FALSAFA DOKTORI (PHD) DISSERTATSIYASI AVTOREFERATI

Toshkent – 2025

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Falsafa doktori (PhD) dissertatsiyasi mavzusi O'zbekiston Respublikasi Oliy ta'lim, fan va innovatsiyalar vazirligiga qarashli Oliy attestatsiya komissiyasida B2024.3.PhD/B1238 raqami bilan ro'yhatga olingan.

Dissertatsiya Oliy ta'lim, fan va innovatsiyalar vazirligi huzuridagi Ilg'or texnologiyalar markazida bajarilgan.

Dissertatsiya avtoreferati uch tilda (o'zbek, ingliz va rus (rezyume)) Ilmiy kengashning veb-sahifasida www.cat-dscphd.uz va «Ziyonet» Axborot ta'lim portalida (www.ziyonet.uz) joylashtirilgan.

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Dissertatsiya himoyasi Oliy ta'lim, fan va innovatsiyalar vazirligi huzuridagi Ilg'or texnologiyalar markazi qoshidagi ilmiy darajalar beruvchi DSc.03/07.2024.B.179.01 raqamli ilmiy kengashning «13» avgust 2024 yil soat 10^{00} dagi majlisida bo'lib o'tadi (Manzil: 100174, Toshkent shahri, Olmazor tumani, Talabalar shaharchasi, Universitet ko'chasi, 3A, Tel.: (99871) 227 43 21

Dissertatsiya bilan Oliy ta'lim, fan va innovatsiyalar vazirligi huzuridagi Ilg'or texnologiyalar markazi qoshidagi Axborot-resurs markazida tanishish mumkin (№ 4 raqami bilan ro'yxatga olingan). (Manzil: 100174, Toshkent shahri, Olmazor tumani, Talabalar shaharchasi, Universitet ko'chasi, 3A, Tel.: (99871) 227 43 21. E-mail: catscience@exat.uz

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KIRISh (Falsafa doktori (PhD) dissertatsiyasi annotatsiyasi)

Dissertatsiya mavzusining dolzarbligi va zarurati. Dunyoda COVID-19 kasalligini oldini olish unga qarshi samarali va xavfsiz vaksinalar ishlab chiqishga alohida e'tibor qaratilmoqda. Vaksinaning maqsadi immunitet tizimini tanib olishi va reaksiyaga kirishi mumkin bo'lgan begona antigen ishlab chiqarishi orqali immunitet reaksiyasini rag'batlantiradi. Shuningdek, platformalarda tirik zaiflashtirilgan vaksinalar kabi an'anaviy usullardan foydalanib, mRNK, virus vektori, oqsil subuniti va virusga o'xshash zarrachalar asosida vaksinalar ishlab chiqishning zamonaviy usullari keng tahlil etilgan. Ta'kidlash joizki, har bir platforma SARS-CoV-2 qarshi global emlashda noyob mexanizmlar hamda afzalliklar va qiyinchiliklarga ega. Oqsil subuniti va virusga o'xshash zarracha vaksinalari bir qator afzalliklarga ega ya'ni xavfsiz, ular yuqumli emas va kasallik keltirib chiqarmaydi, immuniteti zaif odamlar uchun mos keladi. Shu sababli, SARS-CoV-2 virusi tomonida yuzaga kelgan COVID-19 kasalligiga qarshi mahalliy rekombinant oqsil vaksinasi ishlab chiqish muhim ahamiyat kasb etadi.

Jahonda SARS-CoV-2 virusiga qarshi rekombinat oqsil vaksinasi mRNK va vektor vaksinalarini ishlab chiqish hamda joriy etish borasida ilmiy izlanishlar olib borilmoqda. Bu borada, SARS-CoV-2 antigenlarining ekspressiyasi o'rganish, RBD oqsillarini suyuqlik xromatografiya usullari yordamida tozalash, laboratoriya amaliyoti sharoitlarida vaksinaning klinik oldi xavfsizligini taxlil qilish, immunitet tizimiga ta'sirini aniqlash, antitanalarning hosil bo'lishi, sitokinlar darajasi va hujayrali immunitet javobi kabi bir qator parametrlarni tadqiq qilishi, klinik bosqichga o'tishdan oldin har qanday jiddiy nojo'ya ta'sirlarni minimallashtirish, vaksina yaratishda o'ziga xos yondashuvlarni takomillashtirish, shu sababli SARS-CoV-2 virusiga qarshi mahalliy rekombinant oqsil vaksinasini ishlab chiqishni va tahlil qilishni taqozo etmoqda.

Respublikamizda yuqumli kasalliklarning oldini olish, SARS-CoV-2 koronovirusini erta aniqlash va davolash, shuningdek, bemorlarni zarur dori vositalari bilan ta'minlash, import o'rnini bosuvchi dori vositalari hamda mahalliy rekombinant oqsil vaksinalarini ishlab chiqish bo'yicha muayyan natijalarga erishilmoqda. O'zbekiston Respublikasi Prezidentining 2022-2026 yillarga mo'ljallangan yangi O'zbekiston Taraqqiyot strategiyasi[1] "Farmatsevtika sanoati mahsulotlari ishlab chiqarish hajmini 3 barobar ko'paytirish va mahalliy bozorni ta'minlash darajasini 80 foizga yetkazish, import mahsulotlar hajmini va tarkibini optimallashtirish hamda mahalliy ishlab chiqaruvchilarni qo'llab-quvvatlash" vazifalari belgilab berilgan. Ushbu vazifalarni amalga oshirishda SARS-CoV-2 antigenlarining ekspressiyasini aniqlash hamda SARS-CoV-2 virusining RBD fragmentining rekombinant dimer shakliga asoslangan RENOVAC vaksinasini ishlab chiqish va amaliyotga joriy etish muhim ahamiyat kasb etadi.

O'zbekiston Respublikasi Prezidentining 2022 yil 28 yanvardagi PF-60-son "2022-2026 yillarga mo'ljallangan Yangi O'zbekistonning taraqqiyot strategiyasi" to'g'risidagi Farmoni, O'zbekiston Respublikasi Prezidentining 2020 yil 25 iyuldagi PF-6035-son "Koronavirus pandemiyasini yumshatish, sanitariya-epidemiologik

[1] O'zbekiston Respublikasi Prezidentining 2022 yil 28 yanvardagi PF-60-son "2022 -2026 yillarga mo'ljallangan Yangi O'zbekistonning Taraqqiyot strategiyasi to'g'risida" gi Farmoni

osoyishtalik va aholi sog‘lig‘ini himoya qilish tizimini tubdan takomillashtirish chora-tadbirlari to’g‘risida”gi Farmoni, O‘zbekiston Respublikasi Prezidentining 2020 yil 26 martdagi PQ-4649-son “O‘zbekiston Respublikasida koronavirus infeksiyasining keng tarqalishining oldini olish bo‘yicha qo‘shimcha chora-tadbirlar to‘g‘risida”gi qarori, O‘zbekiston Respublikasi Prezidentining 2024 yil 23 yanvardagi PF-20-son “Farmatsevtika sohasini tartibga solish bo‘yicha qo‘shimcha chora tadbirlar to‘g‘risida”gi Farmoni hamda mazkur faoliyatga tegishli boshqa me’yoriy-huquqiy hujjatlarda belgilangan vazifalarni amalga oshirishga ushbu dissertatsiya tadqiqoti muayyan darajada xizmat qiladi.

Tadqiqotning respublika fan va texnologiyalari rivojlanishining ustuvor yo‘nalishlariga mosligi. Ushbu tadqiqot respublika fan va texnikasini rivojlantirishning ustuvor yo‘nalishi – VI "Tibbiyot va farmakologiya"ga muvofiq amalga oshirildi.

Muammoning o‘rganilganlik darajasi. Jahon ilmiy adabiyotlarida koronaviruslarning morfologik tuzilishi, genetik o‘lchami va klassifikatsiyasi bo‘yicha hamda viruslarning turli organizmlarda chaqiradigan infeksion kasalliklar xususiyatlari haqida ma’lumotlar keltirilgan (Woo va boshq., 2010; Cherry va boshq., 2017). Koronaviruslar oilaga kiruvchi viruslarning ayrim turlari odamlarda yengil shamollash alomatlarini keltirib chiqarishi, boshqalari esa og‘ir klinik holatlar, jumladan, respirator sindromlar bilan kechuvchi kasalliklarga sabab bo’lishi, shuningdek, koronaviruslar sigir va cho‘chqalarda diareya, sichqonlarda esa gepatit va ensefalomiyelit kabi infeksion kasalliklarni qo‘zg‘atishi yuzasidan ilmiy tadqiqotlar olib borilgan (Cherry va boshq., 2017; Fan va boshq., 2019). So‘nggi yigirma yil ichida 2 ta zoonotik koronavirus: og‘ir o‘tkir respirator sindromi (SARS), Yaqin Sharq respirator sindromi (MERS) insonlar orasida epidemiyalar tarqalishiga sabab bo‘lganligi va minglab insonlarning hayotiga zomin bo’lganligi aniqlangan (Chan-Yeung va Xu, 2003; Zumla va boshq., 2015).

COVID-19 kasalligining keng tarqalishi virusning kelib chiqishi (Zhou va boshq., 2020), to‘liq genom ketma-ketligi (Fan va boshq., 2020), infeksiya mexanizmlari (Jackson va boshq., 2022; Khreefa va boshq., 2023) hamda virus va uning oqsillarining tuzilishi bo‘yicha ma’lumotlar o‘rganilgan (Huang va boshq., 2020; Yan va boshq., 2022). Ushbu tadqiqotlar SARS-CoV-2 virusiga qarshi maqsadli kurash olib borish imkonini berib, diagnostik to’plamlar (Alhamid va boshq., 2022; El-Daly, 2024) va vaksinalar ishlab chiqish tadqiqotlariga zamin yaratdi. COVID-19 tarqalishini oldini olish maqsadida turli platformalarga asoslangan vaksinalar ishlab chiqilganligi bir qator adabiyotlarda tahlil qilingan (Zhang va boshq., 2022; Rahman va boshq., 2022). COVID-19 ga qarshi ishlab chiqilgan vaksinalar va ularni klinik sinovdan o‘tkazish bo’yicha ma’lumotlar JSST tomonidan keltirib o’tilgan (JSST COVID-19 vaksinalar kuzatuvi, 2025). Barcha mavjud vaksinalar SARS-CoV-2 ning yuzasida joylashgan oqsil va uning fragmentlarini antigen sifatida nishonga olishi, ayniqsa uning ACE-2 retseptoriga bog‘lanish domenlari (RBD) eng istiqbolli nomzod sifatida qaralishi bo’yicha fikrlar keltirilgan (Sheikhshahrokh va boshq., 2020; Dai va Gao, 2021). Xususan, BioNTech/Pfizer va Moderna kompaniyalari tomonidan ishlab chiqarilgan mRNK vaksinalari, organizmga yuborilgandan so‘ng virusning tojdor oqsilini sintez qilishga asoslangan (Polack va boshq., 2020; Baden va boshq., 2021), yurtimizda III

bosqich klinik sinovlari o’tkazilib 84,8% samaradorlik namoyon etgan ZF-2001 vaksinasi koronavirus toj oqsilining RBD antigeni asosida ishlab chiqilgan (Dai va boshq., 2020; Turdikulova va boshq., 2022),

Respublikamiz olimlari tomonidan xususan, Ilg‘or texnologiyalar markazi xodimlari tomonidan SARS-CoV-2 virusini aniqlashda mahalliy diagnostika test-sistemasi yaratilgan (Sh.U.Turdikulova va boshq., 2022; Sh.N.Ibragimova, 2025), SARS-CoV-2 virusining to‘liq genomi Ilg‘or texnologiyalar markazi (A.Abdullaev va boshq., 2022; G.Esonova va boshq., 2024) hamda Genomika va bioinformatika markazi (M.S.Ayubov va boshq., 2022; M.S.Ayubov va boshq., 2024) tomonidan sekvenslanib, mutant variantlarning yurtimizda tarqalish tendensiyalari tahlil qilingan. Pomidor o’simligi mevalarida olingan iste’mol qilish mumkin bo’lgan mahalliy TOMAVAC vaksinasi virusning tojdor oqsili fragmenti asosida ishab chiqilgan (Z.T.Buriev va boshq., 2024). Shuni takidlash joizki, CHO hujayralarida SARS-CoV-2 virusiga qarshi mahalliy rekombinant oqsil vaksina ishlab chiqish hamda uning preklinik xavfsizligi va immun profilini o‘rganish birinchi marta amalga oshirilmoqda.

Tadqiqotning dissertatsiya bajarilgan oliy ta’lim yoki ilmiy-tadqiqot muassasasining ilmiy tadqiqot ishlari rejalari bilan bog’liqligi. Dissertatsiya tadqiqoti Ilg‘or Texnologiyalar Markazi tadqiqot ishlari rejasining M-2021-1 sonli "CHO hayvon hujayralaridan foydalanib anti-SARS-CoV-2 DNK va rekombinant oqsil vaksinalarini ishlab chiqish" (2021-2023) mavzusidagi maqsadli loyihasi doirasida bajarilgan.

Tadqiqotning maqsadi CHO hujayralarida SARS-CoV-2 virusiga qarshi mahalliy rekombinant oqsil vaksinasi ishlab chiqish hamda uning preklinik xavfsizligi va immun profilini aniqlashdan iborat.

Tadqiqotning vazifalari:

SARS-CoV-2 virusi infeksiyasiga qarshi oqsil subbirlik vaksina nomzodini ishlab chiqish;

vaksina nomzodidagi T va B limfotsit epitoplarini in silico bioinformatik vositalar yordamida bashorat qilish;

vaksina nomzodining Sprague Dawley kalamushlarida klinik oldi xavfsizligini baholash;

vaksina nomzodining Balb/c sichqonlarida klinik oldi immun profilini aniqlash.

Tadqiqotning obyekti sifatida antigen sintezlovchi konstruksiyalar, ekspressiya qilingan antigenlar, CHO hujayralari, Sprague Dawley kalamushlari va Balb/c sichqonlaridan foydalanilgan.

Tadqiqotning predmetini vaksina antigenlarini loyihalash va yaratish, ularning CHO hujayralarida ekspressiyasini baholash hamda tayyorlangan vaksina nomzodining xavfsizligi va immunogenligini laboratoriya hayvonlarida aniqlash tashkil etgan.

Tadqiqotning usullari. Tadqiqot jarayonida molekular biologiya, biotexnologiya, bioinformatik dasturlardan, biokimyo, mikrobiologiya va statistik tahlil usullaridan foydalanilgan.

Tadqiqotning ilmiy yangiligi quyidagilardan iborat:

CHO hujayralarida bir qator SARS-CoV-2 antigenlarining ekspressiyasi o‘rganilgan va dimer RBD oqsili tadqiqot sinovidan o‘tgan tojdor oqsillariga nisbatan ko‘proq miqdorda ekspressiyalanishi hamda dimer RBD oqsilining tozalashdan so‘ng unumi 20-50 mg/l, molekular massasi 50-55 kDa atrofida ekanligi, CHO hujayrasida ekspressiyalanganda butun va degradatsiya formasida aniqlangan hamda RBD oqsilini suyuqlik xromatografiya usullari yordamida oqsilning butun formasini 99% gacha tozalashga erishilgan va tozalash protokoli ishlab chiqilgan;

ilk bor gen muhandisligi yordamida CHO hujayralarida olingan rekombinant dimer RBD oqsili asosida ikki xil: 10 μg/doza va 25 μg/doza konsentratsiyadagi RENOVAC vaksinasi ishlab chiqilgan;

RENOVAC vaksinasi Sprague Dawley kalamushlari mushak ichiga 25 μg dozada qo‘llanilganda taloq va timusda kuzatilgan o‘zgarishlar vaksinaga nisbatan immunologik javob bilan bog‘liqligi va toksikologik jihatdan nojo‘ya o‘zgarishlar qayd etilmaganligi hamda vaksinaning nojo‘ya ta’sirlarga sabab bo‘lmaslik darajasi (NOAEL) 25 μg/dozada ekanligi asoslangan;

Balb/c sichqonlariga intraperitonel usulida qo‘llanilishi vaksina past va yuqori dozalarda yaxshi immunogenlik potensialini ko‘rsatgan, lekin 10 mkg/doza vaksina natijasida hosil bo‘lgan anti-RBD va virusni zararsizlantiradigan antitanalar miqdori yetarli bo‘lib, ular ko‘proq vaqt davomida ko‘p miqdorda saqlanishi aniqlangan;

ilk O‘zbekistonda vaksinaning ta’sir mexanizmi aniqlangan, birinchi navbatda CD4 + yordamchi T-hujayralarini faollashtirish orqali immun javobini keltirib chiqarishi va o‘z navbatida ushbu T hujayralarning bu faollashuvi keyinchalik B hujayralarining faollashuvi orqali gumoral immunitetning shakllanishiga vositachilik qilishi, CD8 + hujayralari va interleykin-18 kabi boshqa ko‘rsatkichlardagi o‘zgarishlar vaksinatsiyadan keyin hujayra immunitetini faollashtirishi mumkinligi aniqlangan.

Tadqiqotning amaliy natijalari quyidagilardan iborat:

SARS-CoV-2 virusining RBD fragmentining rekombinant dimer shakliga asoslangan RENOVAC vaksinasi yaratilgan va CAT-Biotech tajriba sinov kompleksida ishlab chiqarilgan;

dimer RBD oqsili alyuminiy gidroksidi bilan birga formulalanib kalamushlar va sichqonlarda ikki xil doza asosida xavfsizlik va immunogenlik tadqiqotlar o‘tkazilgan;

RENOVAC vaksinasi preklinik tadqiqotlarida xavfsizligi va yuqori himoyasi hamda ta’sir mexanizmining ilmiy asoslari aniqlangan.

Tadqiqot natijalarining ishonchliligi zamonaviy molekular biologiya bioinformatika, biokimyo va biotexnologik usullar qo‘llanilganligi, preklinik, yaxshi laboratoriya amaliyoti (GLP) talablariga muofiq, tegishli institutlarning etik komissiyasi ruxsati bilan o‘tkazilishi, natijalar auditdan o‘tkazilishi, sinov va platsebo guruhlari o‘rtasidagi farqlar bir yo‘nalishli ANOVA statistik usuli yordamida hamda platsebo va nazorat guruhlari farqlari t-test tahlilida aniqlanishi bilan isbotlanadi.

Tadqiqot natijalarining ilmiy va amaliy ahamiyati. Tadqiqot natijalarining ilmiy ahamiyati RBD-RENOVAC dimer vaksinasining past dozasi maqsadli IgG ning yuqori darajasini qo‘zg‘atish va sezilarli darajada neytrallash samaradorligi

bilan uzoq muddatli immunitetni ta’minlashi, sutemizuvchilar hujayralaridan foydalanib rekombinant oqsil vaksinalarini ishlab chiqish platformasi yaratilganligi hamda dimerik RBD oqsilining yuqori samaradorligi nafaqat uni ishlab chiqarishni osonlashtirishi balki uning kengaytirilgan vaksina platformasining hayotiy komponenti sifatida potensialini oshirishi, rekombinant oqsilga asoslangan vaksinalarni ishlab chiqish boshqa paydo bo‘ladigan yuqumli kasalliklarga nisbatan qo‘llanilishi bilan xizmat qiladi.

Tadqiqot natijalarining amaliy ahamiyati dimer RBD konstruksiyasiga asoslangan RENOVAC oqsil subunit vaksinasi ishlab chiqilgan hamda uning xavfsizligi va immunprofili SARS-CoV-2 ning oldini olish uchun samarali vaksina nomzodi sifatida tavsiya etgan, O‘zbekistonda ushbu platforma boshqa vaksinalarni ishlab chiqishda, shuningdek, yuqumli kasalliklar, kelajakdagi pandemiyalar va biologik tahdidlarga tayyorgarlikni oshirishga xizmat qiladi.

Tadqiqot natijalarining joriy qilinishi. SARS-CoV-2 virusiga qarshi rekombinant oqsil vaksinasini yaratish va amaliyotga joriy etish jarayonida olingan ilmiy natijalar asosida:

SARS-CoV-2 virusiga qarshi rekombinant oqsil vaksina nomzodi CAT-Biotech tajriba sinov ishlab chiqarish kompleksida kichik partiyada ishlab chiqilgan (O‘zbekiston Respublikasi Oliy ta’lim fan va innovatsiyalar vazirligining 2025 yil 17 martdagi 5/68-son ma’lumotnomasi). Natijada vaksina yaratish davomida ishlab chiqilgan suyuqlik xromatografiya orqali oqsillarni tozalab olish texnologiyasi ishlab chiqish imkonini bergan;

CHO hujayralarida ishlab chiqarilgan eruvchan rekombinant S oqsil hujayralari Jahon sog‘liqni saqlash tashkiloti (JSST) tomonidan COVID-19 ga qarshi vaksinalarni ishlab chiqish platformasida ro‘yxatga olingan (https://www.who.int/teams/blueprint/covid-19/covid-19-vaccine-tracker-and-landscape). Natijada boshqa vaksinalarni ishlab chiqish va solishtirish imkonini bergan;

SARS-CoV-2 koronavirusga qarshi dimer RBD oqsili DNK ketma-ketligi GenBank va National Center for Biotechology Information (NCBI) ma’lumotlar bazasida PV232931 va XPR33336.1 raqamlar ostida ro‘yxatdan o‘tkazilgan (https://www.ncbi.nlm.nih.gov/nuccore/PV232931.1/). Natijada yangi vaksinalarni ishlab chiqish DNK yoki oqsil ketma-ketligini takomillashtirish va o‘zaro taqqoslash imkonini bergan.

Tadqiqot natijalarining aprobatsiyasi. Ushbu dissertatsiya tadqiqotining natijalari 2 ta xalqaro va 4 ta respublika ilmiy-amaliy anjumanlarida taqdim etilgan.

Tadqiqot natijalarining e’lon qilinganligi. Dissertatsiya mavzusi bo‘yicha 7 ta ilmiy ishlar, shu jumladan 3 ta ilmiy maqola bo‘lib, Respublikasi Oliy attestatsiya komissiyasi tomonidan dissertatsiyalarning ilmiy natijalarini chop etish uchun tavsiya etilgan nufuzli ilmiy jurnallarda jumladan, 1 tasi respublika nashrlarida, 2 tasi xorijiy jurnalda nashr etilgan.

Dissertatsiyaning tuzilishi va hajmi. Dissertatsiya ishi kirish, 3 bob, xulosa, foydalanilgan adabiyotlar ro‘yxati va ilovalardan iborat. Dissertatsiya hajmi 97 bet.

DISSERTATSIYANING ASOSIY MAZMUNI

Kirish qismida mavzuning dolzarbligi va zarurati asoslab berilgan, tadqiqotning maqsadi va vazifalari aniqlangan, obyekti va predmeti tavsiflangan. Shuningdek, tadqiqotning respublika fan va texnologiyalari rivojlanishining ustuvor yo‘nalishlariga muvofiqligi ko‘rsatilgan. Ishning ilmiy yangiligi va amaliy natijalari bayon qilinib, olingan natijalarning ilmiy hamda amaliy ahamiyati yoritilgan. Tadqiqot natijalarining amaliyotga joriy etish istiqbollari, nashr etilgan ilmiy ishlar va dissertatsiya tuzilishi haqidagi ma’lumotlar keltirilgan.

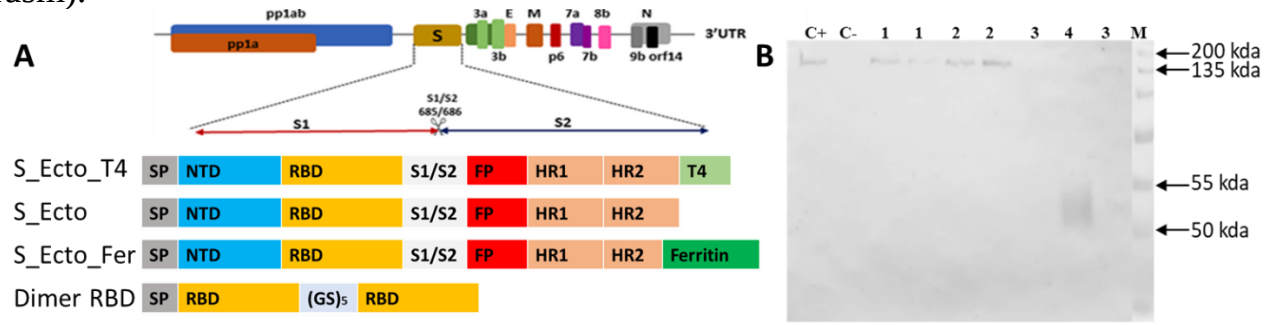
Dissertatsiyaning **“COVID-19 pandemiyasi va infeksiyani oldini olish uchun vaksinalarni ishlab chiqish”** deb nomlangan birinchi bobida COVID-19 pandemiyasining butun dunyo va O‘zbekistonga ta’siri, virusning tuzilishi, evolutsiyasi va patogenezi tahlil qilinib, vaksinalarning tarixi va vaksinatsiya dasturlarining ahamiyati chuqur yoritilgan. Ushbu bobda COVID-19 pandemiyasini nazorat qilish uchun qo‘llanilgan vaksina platformalari, xususan, butun organizm vaksinalar va rDNK texnologiyalariga asoslangan vaksina platformalari tahlil qilinib, yaratilgan nomzod vaksinalarning o‘ziga xosligi va platformalarning ustunlik va kamchiliklari chuqur tahlil qilingan. Shuningdek, bob so‘ngida vaksinalarni ishlab chiqish bosqichlari va COVID-19 pandemiyasi davrida qo‘llanilgan rivojlanish bosqichlari haqida ma’lumotlar berilgan.

Dissertatsiyaning **“Vaksinalarni ishlab chiqish va preklinik baholashda qo‘llanilgan materiallar va usullar”** deb nomlangan ikkinchi bobida tadqiqot olib borish bosqichlari, ularning bajarilishida foydalanilgan materiallar va uslublar keltirilgan. Bob asosiy vazifalarga mos ravishda, 3 ta yirik qismlardan tashkil topgan bo‘lib, birinchi qismida rekombinant oqsil vaksinani ishlab chiqishda qo‘llanilgan tadqiqot usullari, xususan, oqsil antigenlarni dizayn qilish, ekspression vektorlarni yaratish, vektorlarni tayyorlash, antigenlarni CHO hujayralaridagi ekspressiyasi, dimer RBD oqsilidagi epitoplarning bioinformatik tahlili hamda suyuqlik xormatografiyasi metodlari yordamida tozalab olish usullari keltirilgan. Shunindek, ushbu jarayonda qo‘llanilgan rutin analiz protokollari ham keltirib o‘tilgan. Bobning keyingi ikkinchi va uchinchi qismlarida, mos ravishda preklinik xavfsizlik va immun profilini o‘rganish jarayoni to‘liq yoritilgan. Jumladan, laborator hayvonlar tadqiqotning etik aspektlari, tadiqot dizayni hamda belgilangan parametrlarni o‘rganish davomida qo‘llanilgan protokollar to‘liq yoritilgan. Bob so‘ngida esa guruhlarni o‘zaro solishtirish uchun qo‘llanilgan statitk dasturlar va tajlil usullari, xususan, ANNOVA va t test usullarining qanday olib borilgani yoritilgan.

Dissertatsiyaning **“Vaksina ishlab chiqish va preklinik baholash natijalari va muhokamasi”** nomli uchinchi bobida tadqiqot vazifalariga mos ravishda birinchi qismda vaksinani ishlab chiqish, bosqichi natijalari keltirilib, tahlil qilingan. Ikkinchi qismida esa vaksinaning Sprague Dawley kalamushlarida xavfsizlik profili baholash uchun o‘tkazilgan tadqiqotlar natijalari va tahlili keltirilgan. Nihoyat, uchinchi qismida Balb/c sichqonlarida o‘tkazilgan immun profil analizi natijalari keltirilib, tahlil qilingan.

Tadqiqot davomida SARS-CoV-2 tojdor oqsili va uning RBD fragmenti asosida 4 xildagi vaksina antigenlar dizayn qilinib, pcDNA3.4 plazzmidasiga XbaI

va AflII restriksion fermentlari yordamida CMV promotori ostiga klonlandi. (1A-rasm).



1-rasm. Yaratilgan antigen kosntruksiyalari hamda ularning ekspressiya analizi. A – antigenlarning tuzilishi; B – Antigenlarning CHO hujayralardagi eskpressiya analizi: 1 – S_Ecto_T4, 2 – S_Ecto, 3 – S_Ecto_Fer, 4 – Dimer RBD.

S_Ecto_T4: Spike ektodomenini kodlaydi (aminokislotalar 13-1213) va trimerning barqaror shakllanishiga yordam berish uchun C-terminal qismida T4 foldon trimerizatsiya domenini kodlaydi. **S_Ecto:** Oldingi konstruktsiyaga o'xshash, lekin T4 foldon ketma-ketligi yo'q, bu trimerizatsiya domenining ekspressiya va antigen barqarorligiga ta'sirini taqqoslash imkonini beradi. **S_Ecto_Fer:** Kesilgan HR2 fragmenti va *H.pylori* ferritin multimerizatsiya oqsili o'z ichiga oladi. Multimerlarni shakllantirish orqali antigen taqdimotini kuchaytirishga qaratilgan. **Dimer RBD:** Ushbu konstruksiya antigen zichligini oshirish va immunitetni tanib olishni kuchaytirish uchun 5xGS bog'lovchisi bilan bog'langan tandemli takrorlangan RBD ketma-ketligini kodlaydi va ushbu konstruktsiyaning o'zgartirilgan versiyasi tozalashni osonlashtirish uchun maqsadli oqsilning C terminal tomonida 6ta gistidin nishonini qo'shimcha ravishda kodlaydi.

Ko‘plab tadqiqotlar SARS-CoV-2 tojdor oqsili va uning fragmentlarini rekombinant texnologiyalari asosida samarali ekspressiyalash mushkul ekanligini ko‘rsatdi. Shu sababdan ham dastlabki tadqiqotda yaratilgan konstruksiyalarning CHO hujayralaridagi ekspressiyasi baholanib, dimer RBD oqsili boshqa oqsillarga nisbatan samaraliroq ekspressiya bo‘lishi aniqlandi (1B-rasm).

Dimer RBD oqsili ekspressiyasi boshqa konstruksiyalarga nisbatan yuqori ekanligi sababli ushbu konstruksiya vaksinani ishlab chiqish uchun tanlab olindi hamda keyingi izlanishlar davomida bioinformatik dasturlar asosida nomzod antigen tarkibidagi MHCI/II T hujayra epitoplari bashorat qilinib, oqsil tarkibidagi RLFRKSNLK MHCI epitopi, nafaqat turli HLA allellari uchun mos kelishi balki empirik tadqiqotlarda tasdiqlangan va eksperimental tahlillarda T hujayralarini samarali faollashtirishi ko'rsatilgan. Ushbu turdagi epitoplar sitotoksik CD8+ T hujayralarining reaktsiyalarini keltirib chiqarish, va infeksiyalangan hujayralarni nishonga olish uchun juda muhimdir. MHCII eptiroplari esa antitan ishlab chiqarishni qo'llab-quvvatlaydigan va kengroq immunitet reaktsiyalarini muvofiqlashtiradigan CD4 + yordamchi T hujayralarini faollashtiradi. Dimer RBD tarkibidagi FERDISTEI va FASVYAWNR kabi eptioplar bir nechta HLA allellari bilan kuchli bog‘lanish ehtimolligi mavjudligi aniqlandi

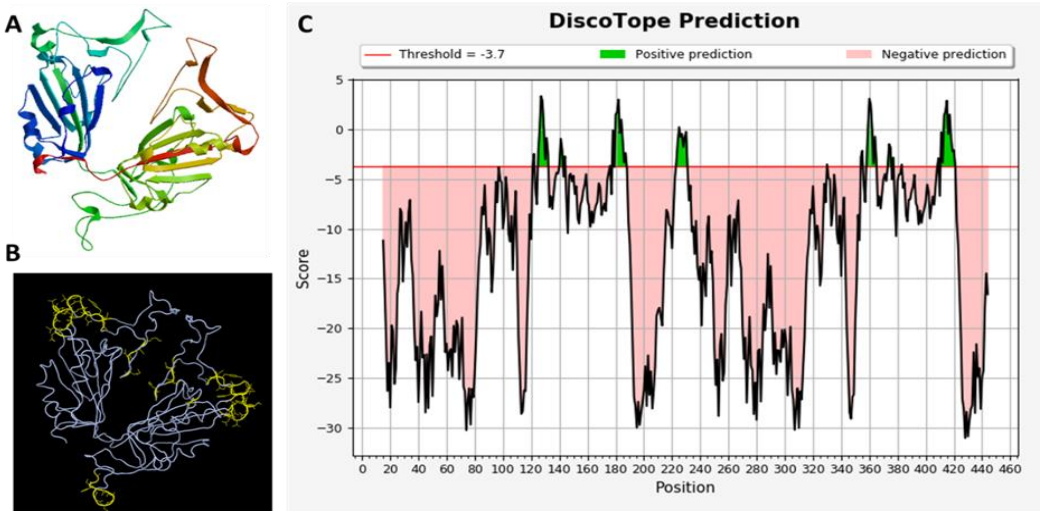
Dimer RBD tarkibidagi bashorat qilingan 10 ta chiziqli B hujayra epitoplari adabiyotlarda ilgari tasdiqlangan epitoplar bilan yuqori darajada o'xshashligi aniqlandi (1-jadval).

1-jadval.

Tasdiqlangan B hujayra chiziqli epitoplar bilan mos keladigan bashorat qilingan Dimer RBD epitoplari.

№	Start	Stop	Bashoratlangan epitope amino kislota sekvensi	Spike oqsilidagi pozitsiyasi
1.	26	45	ATRFASVYAWNRKRISNCVA	344-363
2.	55	60	SFSTFK	373-378
3.	86	109	GDEVRQIAPGQTGKIADYNYKLPD	404-427
4.	122	188	NLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQ AGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQ	440-506
5.	201	216	HAPATVCGPKKSTNLV	519-534
6.	258	276	NATRFASVYAWNRKRISNC	343-361
7.	318	342	RGDEVRQIAPGQTGKIADYNYKLPD	403-427
8.	355	382	NLDSKVGGNYNYLYRLFRKSNLKPFERD	440-467
9.	384	420	STEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGV GY	469-504
10.	434	452	HAPATVCGPKKSTNLVKNK	519-537

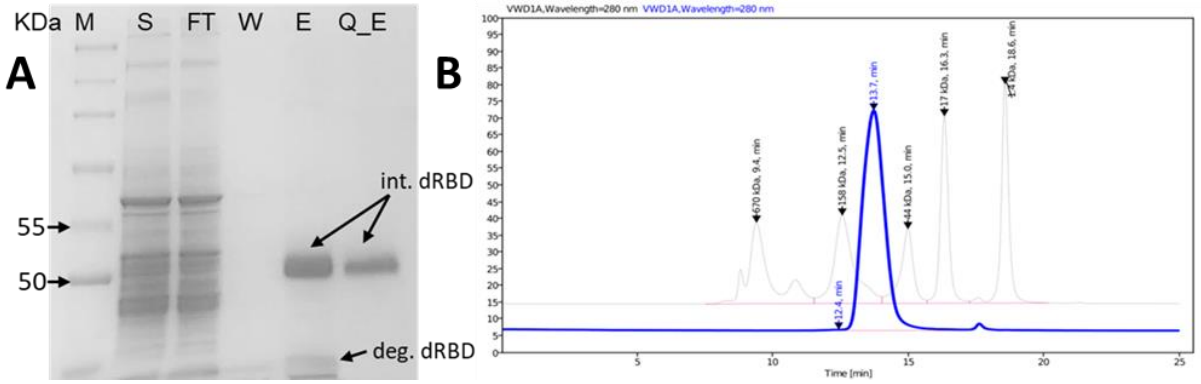
Konformtasion B hujayra epitoplarining bioinformatik tahlili Dimer RBD oqsilida avval tasdiqlangan immunologik ahamiyatga ega bo‘lgan hududlar bilan mos keladigan bir nechta epitoplar mavjudligini tasdiqladi (2-rasm). Ushbu aniqlangan epitoplardan, 416-469 qoldiqlari, ACE2 retseptorlari bilan bog‘lanish uchun muhim segment bo‘lib, K417 kabi muhim qoldiqlarni o‘z ichiga oladi. 441-463 oralig‘idagi epitrop esa ACE2 o‘zaro ta’siri uchun muhim bo‘lgan kalit halqani o‘z ichiga oladi. Nihoyat, 495-506 oralig‘idagi epitop N501 atrofida joylashgan retseptorlarni bog‘laydigan va retseptor bilan bog‘lanish uchun muhim qoldiq.



2-rasm. Dimer RBD vaksina nomzodining taxmin qilingan 3D tuzilishi va konformatsion B hujayra epitoplari. (A) Dimer RBD oqsilining bashorat qilingan 3D modeli. (B) 3D model ko'rinishida dimer RBD vaksinasida bashorat qilingan konformatsion B-hujayra epitoplarining

vizualizatsiyasi. (C) Dimer RBD nomzodida bashorat qilingan konformatsion B-hujayra epitoplari.

CHO hujayralarida ekspressiyalanganda dimer RBD butun va parchalangan holatda ekanligi aniqlandi (3A-rasm). Butun dimer RBD oqsilining molekulyar massasi kutilgani kabi ~ 50-55 kDa bo‘lib, uning ehtimol fermentativ parchalanishi tufayli monomer formadagi RBD qoldiqlari tufayli parchalangan formasi hosil bo‘lgan bo‘lishi mumkin.



3-rasm. Dimer RBD oqsilini tozalash va tozalik darajasini aniqlash. A. Tozalangan Dimer RBD fraksiyalarining PAAG tahlili; S - dastlabki kultura suyuqligi; FT – affin sorbent bilan bog'lanmagan oqsil fraksiyasi; W - 40 mM imidazolni o'z ichiga olgan bufer bilan fraksiyani yuvish; E - maqsadli fraksiyani 250 mM imidazolni o'z ichiga olgan bufer bilan elyutsiyasi; Q_E - ion almashinuv kolonkasidan maqsadli oqsilning elutsiyasi. C. Tozalangan antigenning SEC-HPLC tahlili.

Ikki bosqichli nikel affin hamda ion almashinuv suyuqlik xromatorafiya usulida tozalangan dimer RBD tozaligi kamida 99% ekanligi SEC-HPLC tahlili yordamida tasdiqlandi (3B-rasm). Shuningdek, toza dimer RBD miqdori har bir litr hujayra kulturasi uchun taxminan 20-50 mg ekanligi aniqlandi.

Klinikadan oldingi tadqiqotlar uchun uch turdagi vaksina formulasi – platsebo ya’ni vaksina antigeni tutmagan nazorat, past dozali (har bir dozada 10 µg antigen) va yuqori dozali (har bir dozada 25 µg antigen) - yordamchi vosita sifatida alyuminiy gidroksid (alum) yordamida aseptik tarzda qadoqlanib tayyorlandi hamda RENOVAC nomi ostida ishlab chiqildi.

Tadqiqot vazifalariga mos tarzda, preklinik tadqiqotlar olib borilib, dastlab Sprague Dawley kalamushlarida 7 kun interval bilan jami 2 dozadan tayyorlangan 3 turdagi sinov vaksinalari ineksiyalanib, 14 kunlik toksilik (G1 – platsebo, G2 – past doza vaksina va G3 – yuqori doza vaksina) hamda 14 kunlik toksiklik 28 kun tiklanish davri (G4R – platsebo va G5R – yuqori doza vaksina) bilan **vaksinaning xavfsizlik profilini aniqlash bo‘yicha** tadqiqotlar olib borildi.

Tadqiqot davomida platsebo, past dozali va yuqori dozali RENOVAC vaksinasini qo‘llanilgan kalamushlarda o‘lim yoki g‘ayritabiiy klinik simptomlar kuzatilmadi va muntazam baholashlar davolanish bilan bog‘liq anormalliklarni aniqlamadi, bu emlash xavfsizligini tasdiqlaydi va klinik jihatdan ahamiyatli ko‘rinish, sog‘liq holati yoki xatti-harakatida hech qanday o‘zgarishlar yo‘qligini tasdiqlaydi.

Barcha nazorat va davolash guruhlaridagi erkak va urg‘ochi kalamushlarda tana vaznining odatiy va kutilgan o‘sishi kuzatildi. Bu hayvonlarning normal o‘sishi va rivojlanishini ko‘rsatadi va RENOVAC vaksinasi umumiy salomatlikka hech qanday salbiy ta’sir ko‘rsatmaganligini ko‘rsatadi. Shu bilan birga, tana vaznining dozaga bog‘liq bo‘lgan bir oz pasayishi dastlabki uchta guruh uchun 14-kuni va tiklanish guruhlarida 42-kuni kuzatildi. Ushbu o‘zgarishlar statistik ahamiyatga ega emas va natijada ushbu holat emlash bilan bog‘liq emasligini ko‘rsatadi.

Gematologik tadqiqot natijalari asosiy parametrlarning aniq, lekin toksik ta’sirga ega bo‘lmagan o‘zgarishlarini ko‘rsatdi, bu esa nojo‘ya ta’sirlardan ko‘ra immunologik javoblarni aks ettiradi (2-jadval). Masalan, erkaklarda (ayniqsa, G3 guruhida) 15-kuni oq qon hujayralari (WBC) miqdorining doza bog‘liq oshishi va 43-kuni biroz pasayishi kuzatildi, shuningdek, faqat G2 guruhidagi urg‘ochilari 15-kuni o‘rtacha gemoglobin miqdor (MCH) ning statistik jihatdan ahamiyatli oshishi qayd etildi. Gemoglobin miqdori (HGB), gematokrit darajasi (HCT) va trombositlar miqdori (PLT) darajalaridagi minimal, doza bog‘liq bo‘lmagan o‘zgarishlar ham fiziologik o‘zgaruvchanlikni ko‘rsatib, vaksina bilan bog‘liq toksiklik mavjud emasligini tasdiqladi.

2-Jadval. RENOVAC vaksinasidan keyingi kalamushlarda gematologiya parametrlari

Parametrlar		**Erkak**					**Urg’ochi**				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
WBC (10^3/µL)	Mean	15.97	16.13	19.02	13.68	10.08	12.63	12.43	12.50	7.07	8.12
	SD	3.72	6.43	5.06	3.80	3.09	4.16	4.16	5.67	1.78	1.06
HGB (g/dL)	Mean	12.65	13.07	12.62	13.38	13.92	12.05	12.50	12.55	12.52	12.92
	SD	0.52	0.45	0.52	0.97	1.08	0.61	0.50	0.82	0.74	0.36
HCT (%)	Mean	39.68	40.35	39.27	42.90	44.95	35.55	36.17	37.12	39.13	39.33
	SD	1.61	2.02	1.83	3.11	3.83	1.82	1.32	2.36	2.43	0.96
MCH (pg)	Mean	17.23	17.95	17.28	16.53	16.28	18.25	18.97*****	18.00	17.03	17.53
	SD	0.78	0.92	0.71	0.60	0.69	0.40	0.39	0.67	0.52	0.50
PLT (10^3/µL)	Mean	1067.3	969.3	999.1	801.0	944.3	1023.6	978.0	957.3	870.1	858.5
	SD	156.36	103.71	156.21	160.22	123.38	121.65	116.44	181.60	86.62	120.65

Eslatma: N = 6; Mean = o’rtacha qiymat; SD = Standart og'ish. G1-G3 15-kun, G4R-G5R 43-kun.
Kalit: * = Guruhning o'rtacha qiymati p<0,05 da platsebo nazorat guruhidan sezilarli darajada farq qiladi.

15-kuni differensial leykotsitlar miqdori G3 guruhidagi erkaklarda doza bog‘liq ravishda statistik jihatdan ahamiyatli bo‘lgan neytrofillar sonining oshishi va limfotsitlar sonining nisbiy kamayishini ko‘rsatdi (3-jadval), bunda asosiy tiklanish guruhi urg’ochi va erkaklarida leykotsitlar miqdori tegishli nazorat guruhlari bilan taqqoslanganda sezilarli farq qilmadi; bu gematologik o‘zgarishlar, ayniqsa WBC va neytrofillarning dastlabki oshishi, vaksinatsiyadan keyin kutilgan tug‘ma immun javobiga mos keladi, chunki neytrofillar erta patogenlarga qarshi himoyada va moslashuvchan immunitet shakllanishida muhim rol o‘ynaydi. Limfotsitlar ulushining vaqtinchalik kamayishi esa dastlabki neytrofil ko‘payishi bilan bog‘liq bo‘lib, immun tizimining erta faollashuv bosqichida tabiiy dinamik o‘zgarishlarini

aks ettiradi , bu esa ushbu o‘zgarishlar vaksinaning toksik yoki nojo‘ya ta’siri emas, balki tipik immun javob ekanligini tasdiqlaydi.

3-jadval. RENOVAC vaktsinasidan keyin kalamushlarda differentsial leykotsitlar soni (%)

Parametrlar		Erkak					Urg’ochi				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
Neytrofillar	Mean	22.33	24.00	27.50*****	23.17	24.33	24.67	23.50	25.67	25.50	25.33
	SD	2.25	2.83	3.62	3.37	2.16	3.67	2.81	3.01	2.66	1.63
Limfotsitlar	Mean	76.17	74.33	71.33*****	75.67	74.17	74.00	75.00	73.00	73.17	73.17
	SD	1.94	2.73	3.27	3.61	2.99	4.20	2.37	3.10	2.79	2.32

Eslatma: N = 6; Mean = o’rtacha qiymat; SD = Standart og'ish. G1-G3 15-kun, G4R-G5R 43-kun.
Kalit: * = Guruhning o'rtacha qiymati p<0,05 da platsebo nazorat guruhidan sezilarli darajada farq qiladi.

Vaksinatsiyadan keyin o‘tkazilgan klinik kimyo tahlillari fiziologik va metabolik moslashuvlarni ko‘rsatadigan dinamik, dozaga bog‘liq o‘zgarishlarni aniqladi (4-jadval). 15-kuni erkak va urg‘ochi jins vakillarida glyukoza (GLU) darajasining dozaga bog‘liq oshishi kuzatildi. Shu bilan birga, erkaklarda ishqoriy fosfataza (ALP) va urg‘ochilarda glutamat oksaloatsetat transaminaza (GOT) darajalari sezilarli darajada oshdi, bu esa yengil va vaqtinchalik jigar stressini ko‘rsatdi. Ushbu o‘zgarishlar toksikologik ta’sir emas, balki odatiy vaksina ta’sirida yuzaga keladigan metabolik, jigar va buyrak moslashuvlariga mos keladi.

4-jadval. RENOVAC vaksinasidan keyin kalamushlarda klinik kimyoni baholash.

Parametrlar		Erkak					Urg’ochi				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
GPT (U/L)	Mean	55.58	58.53	59.88	52.10	36.03*	53.23	59.12	51.88	49.37	43.72
	SD	13.29	7.35	10.67	10.41	5.10	10.38	9.93	7.52	9.10	8.09
GOT (U/L)	Mean	104.73	97.38	111.77	84.57	91.60	83.33	99.13	121.07*	93.15	108.17
	SD	14.69	26.71	21.05	8.00	12.21	11.98	17.58	39.14	12.69	24.16
ALP (U/L)	Mean	341.83	509.83*	453.67	388.17	313.67	251.83	262.17	328.67	220.50	239.67
	SD	26.83	148.68	100.77	106.15	70.60	65.24	73.20	54.77	93.13	71.01
BUL (mg/dL)	Mean	34.47	29.22*	34.12	27.55	36.05*	32.23	34.68	36.22	34.52	37.68
	SD	4.81	1.35	3.99	4.63	3.18	6.15	4.28	6.66	2.41	6.08
GLU (mg/dL)	Mean	97.10	107.78	115.25	142.62	115.28	101.52	104.13	113.83	91.87	111.92*
	SD	8.16	22.64	17.58	30.00	13.56	14.11	10.85	17.70	13.04	6.72
CHOLE (mg/dL)	Mean	60.50	65.50	64.33	53.83	53.00	71.00	71.67	66.50*	76.17	71.83
	SD	3.73	5.61	9.93	8.66	5.90	12.49	11.22	6.50	10.21	7.94
TRIG (mg/dL)	Mean	89.52	67.75	71.12	59.17	39.07*	56.87	50.98	45.95	33.45	29.53
	SD	19.77	12.65	26.33	13.56	15.78	19.36	22.28	15.21	8.32	9.18
ALB/GLO	Mean	0.68	0.67	0.64	0.62	0.68*	0.66	0.71	0.73	0.73	0.67
	SD	0.06	0.06	0.06	0.04	0.05	0.06	0.06	0.06	0.03	0.08
CBUN (mg/dL)	Mean	16.08	13.63*	15.92	12.86	16.82*	15.04	16.19	16.90	16.11	17.59
	SD	2.25	0.63	1.86	2.16	1.48	2.87	2.00	3.11	1.12	2.84
K (mmol/L)	Mean	4.68	4.65	4.68	4.43	4.66	4.32	4.39	4.55	4.48	3.91*
	SD	0.25	0.45	0.31	0.32	0.32	0.48	0.13	0.57	0.28	0.12
Cl (mmol/L)	Mean	100.88	105.28	100.27	101.77	102.05	105.18	101.55	101.47	103.13	100.77*
	SD	1.17	9.77	0.98	0.57	1.12	8.57	0.95	1.08	0.74	1.00

Eslatma: N = 6; Mean = o’rtacha qiymat; SD = Standart og'ish. G1-G3 15-kun, G4R-G5R 43-kun.
Kalit: * = Guruhning o'rtacha qiymati p<0,05 da platsebo nazorat guruhidan sezilarli darajada farq qiladi.

Urg‘ochilarda xolesterin (CHOLE) darajasining pasayishi esa immun faollashuv jarayonida lipid almashinuvining o‘zgarishi bilan bog‘liq bo‘lishi mumkin. 43-kuniga kelib, klinik parametrlarning aksariyati boshlang‘ich darajaga qaytdi, bu esa tizimli tiklanish jarayonini aks ettirdi. Ayniqsa, erkaklardagi triglitserid (TRIG) va alanin transaminaza (GPT) darajalarining pasayishi jigar faoliyatining tiklanganini ko‘rsatadi, urg‘ochilarda esa kaliy (K) va xlorid (Cl) darajalarining biroz pasayishi klinik ahamiyatga ega bo‘lmagan yengil elektrolit o‘zgarishlarini bildiradi. Tiklanish guruhlarida BUL va albumin-globulin (ALB) nisbatining oshishi oqsil almashinuvining tiklanishini aks ettiradi. Umuman olganda, ushbu natijalar kuzatilgan metabolik o‘zgarishlar kutilgan immunologik javobning bir qismi ekanligini tasdiqlaydi.

15-kunda o‘tkazilgan siydik tahlili erkak va ayollarda siydikning o‘ziga xos zichligi pasayganini va pH darajasi oshganini ko‘rsatdi, biroq bu o‘zgarishlar faqat urg‘ochilarda statistik ahamiyatga ega ekanligi aniqlandi. Tiklanish davrida esa har ikkala parametr ham nazorat guruhlaridagi ko‘rsatkichlarga mos keldi. Boshqa barcha siydik tahlili parametrlarida sezilarli farq kuzatilmadi va histopatologik bog‘liqlik aniqlanmadi. Guruhlarda o‘tkazilgan umumiy patologik tekshiruv natijasida nazorat guruhlariga nisbatan patologik ahamiyatga ega bo‘lgan hech qanday shikastlanish aniqlanmadi.

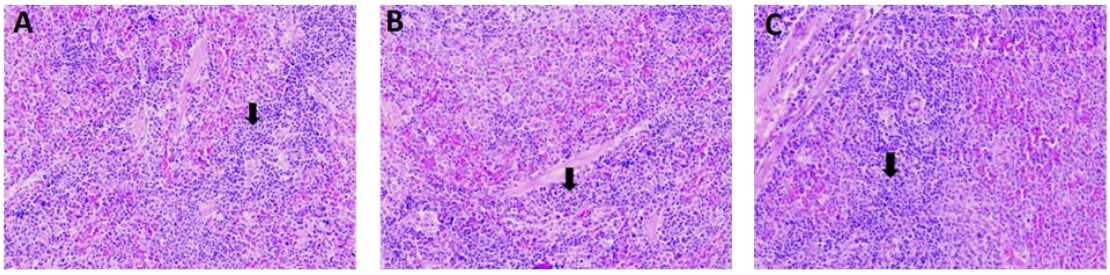
Jarrohlik-patologik tekshiruvdan so‘ng, jigar, buyraklar, buyrak usti bezlari, moyaklar/tuxumdonlar, timus, taloq, miya va yurak kabi organlar tozalanib, vazni o‘lchandi va absolyut hamda nisbiy vazn o‘zgarishlari tahlil qilindi, natijalarda immun javob bilan bog‘liq bo‘lgan taloq kattalashishi, T-hujayralar reaksiyasi bilan bog‘liq timus vaznining o‘zgarishi hamda urg’ochilarda nisbiy yurak va miya vaznining oshishi kabi vaqtinchalik va zararli bo‘lmagan o‘zgarishlar kuzatildi (5-jadval). Aksariyat o‘zgarishlar 43-kunga kelib tiklandi va gistopatologik o‘zgarishlar aniqlanmagani tufayli ular fiziologik jarayonlar bilan bog‘liq deb baholandi.

5-jadval. RENOVAC vaksinasi qo'llanilgandan keyin kalamushlarda terminal tana vazniga nisbatan organ og'irligi

Organ/guruhlar		Erkak					Urg‘ochi				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
Jigar	Mean	4.43	4.27	4.44	4.58	4.01*****	4.03	4.10	4.09	4.21	4.42
	SD	0.50	0.13	0.25	0.46	0.19	0.41	0.45	0.20	0.72	0.54
Yurak	Mean	0.37	0.37	0.36	0.36	0.35	0.35	0.40*****	0.38	0.37	0.38
	SD	0.02	0.03	0.02	0.03	0.02	0.03	0.03	0.02	0.03	0.03
Timus	Mean	0.12	0.10	0.11	0.10	0.11	0.15	0.14	0.16	0.13	0.18*****
	SD	0.03	0.03	0.03	0.03	0.02	0.05	0.02	0.05	0.02	0.03
Miya	Mean	0.79	0.72	0.76	0.72	0.67	0.88	0.95*	0.91	0.89	0.93
	SD	0.07	0.04	0.07	0.13	0.07	0.06	0.04	0.02	0.09	0.16

Eslatma: N = 6; Mean = o‘rtacha qiymat; SD = Standart og'ish. G1-G3 15-kun, G4R-G5R 43-kun.
Kalit: * = Guruhning o'rtacha qiymati p<0,05 da platsebo nazorat guruhidan sezilarli darajada farq qiladi.

Gistopatologik tahlil natijalariga ko‘ra, hech qanday organda muolaja bilan bog‘liq sezilarli o‘zgarishlar aniqlanmadi, faqat G3 guruhidagi ikki erkak va bir ayolda taloqning minimal follikulyar giperplaziyasi kuzatildi (4-rasm).

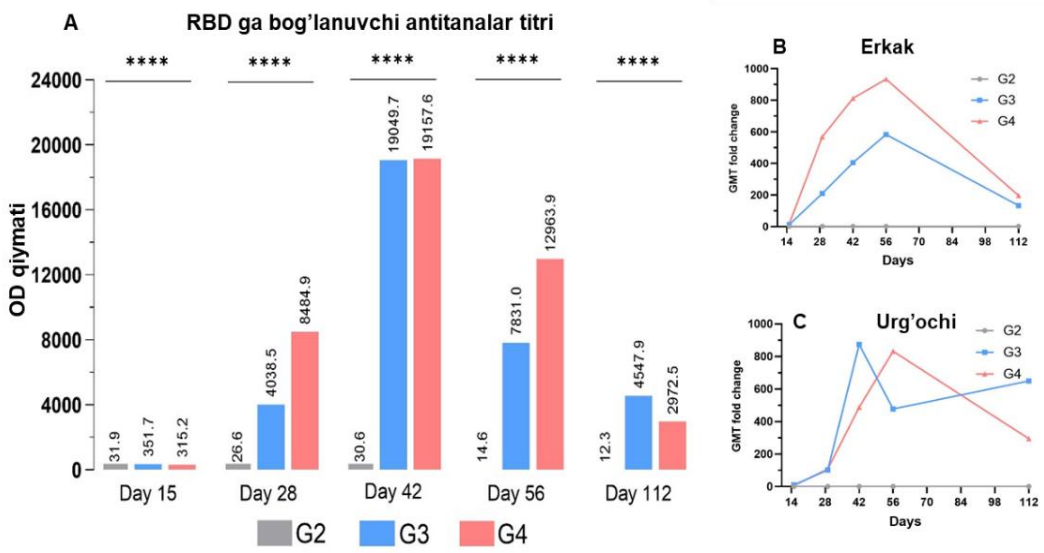


4-rasm. Follikulyar hujayra giperplaziyasining shakllanishi (strelkalar). A va B - G3 erkaklar va C - G3 urg'ochi.

Muhimi, past doza olgan va tiklanish bosqichidagi guruhlarning taloqlari gistologik jihatdan tegishli nazorat guruhlari bilan taqqoslanganda o‘xshash edi, bu esa kuzatilgan o‘zgarishlarning vaqtinchalik va doza bog‘liq ekanligini ko‘rsatadi.

Tadqiqot so’ngi vazifasiga mos tarzda, preklinik tadqiqotlar davom ettirilib, Balb/c sichqonlarida vaksinaning immun profili tadqiq qilindi. Buning uchun 14 kun interval bilan 3 dozada guruhlarga mos ravishda tuzli fosfat buferi - PBS (G1), platsebo (G2), past (G3) hamda yuqori (G4) dozadagi vaksina intraperitoneal yo’l bilan ineksiyalanib, turli davrlarda RBD oqsiliga qarshi spetisifik antitanalarning hosil bo’lishi, virusni neytrallovchi antitanalarning hosil bo’lishi, hujayra hamda sitokin profilining o’zgarishi tadqiq qilindi.

Alyuminiy gidroksid bilan formulalangan dimer RBD vaksinasi past dozali (G3) va yuqori dozali (G4) guruhlarida barcha vaqt oralig‘ida yuqori darajadagi maxsus IgG antitanalar hosil bo‘lishiga olib keldi. Fosfat buferi guruhida anti-RBD IgG va neytrallovchi antitanalar darajasi deyarli aniqlanmadi, shuningdek, platsebo va PBS nazorat guruhlari orasida statistik jihatdan sezilarli farqlar kuzatilmaganligi sababli, aniqroq natijalarni ta’minlash maqsadida PBD guruhi grafiklardan olib tashlandi. Har bir vaksina yuborilgandan so‘ng anti-RBD IgG optik zichlik (OD) qiymatlari oshib borib, uchinchi doza yuborilgandan so‘ng (42-kuni) maksimal darajaga yetishi va keyinchalik 112-kungacha sezilarli darajada pasayishi aniqlandi (5A-rasm).

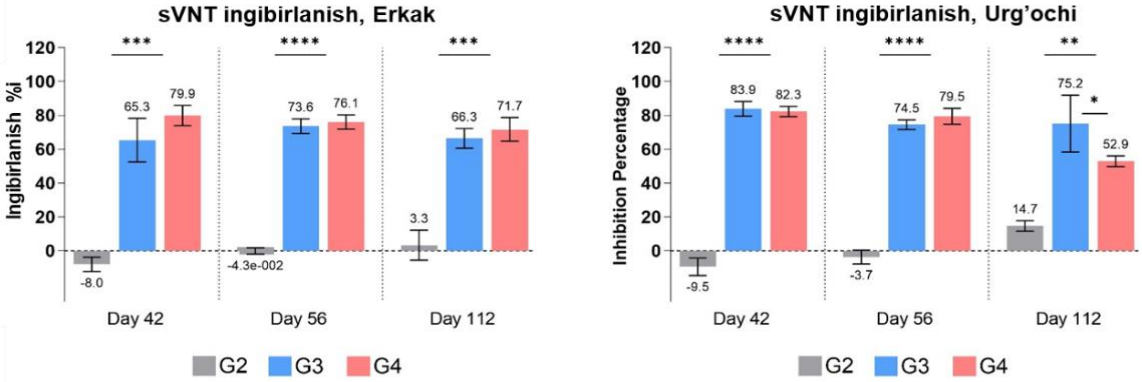


5-rasm. Erkak va urg'ochi sichqonlarda serologik RBDga bog'lanuvchi IgG GMT qiymatining o'zgarishi. P-qiymatlari bir tomonlama ANOVA bilan tahlil qilindi (****p <0.0001).

Geometrik o‘rtacha titr (GMT) tahlili jinsga bog‘liq farqlarni ko‘rsatdi, ya’ni erkaklarda GMT o‘sishi vaksina dozasiga mutanosib bo‘lgan bo‘lsa, urg’ochi sichqonlarda aksincha, 10 µg doza (G3) ga kuchliroq javob qayd etildi. Past dozali

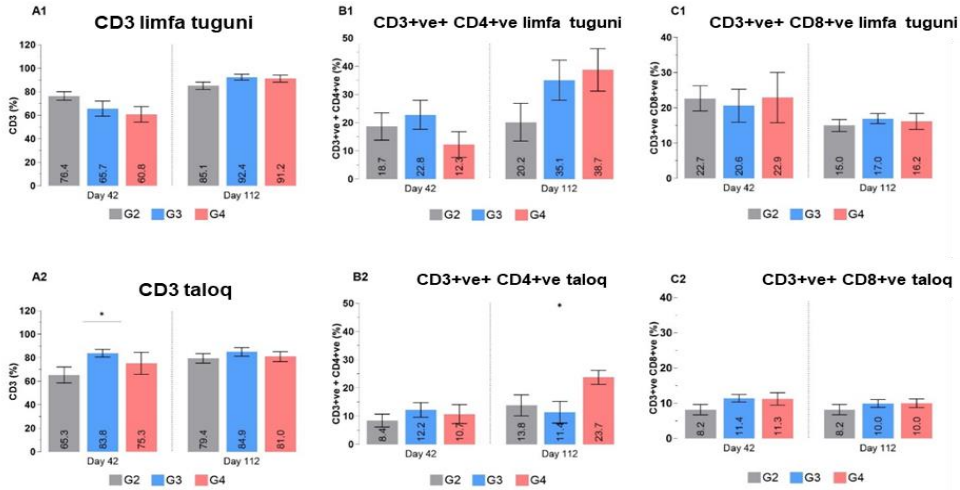
guruhdagi ayollarda esa antitana titri uzoqroq saqlanib, bu ularda erkaklarga qaraganda kuchliroq antitana javobi mavjudligini ko‘rsatgan.

Surrogat SARS-CoV-2 virusining neytrallash analizi natijalari, emlashdan so‘ng hosil bo‘lgan antitanalar SARS-CoV-2 antigen konyugatiga bog‘lanib, uni yuqori darajada ingibirlashi mumkinligini ko’rsatdi (6-rasm). Ikki xildagi dozada ham erkak va urg’ochi sichqonlar yuqori neytrallash faolligini ko‘rsatdi, nazorat guruhlari esa sezilarli darajada ingibirlash qobilyatiga ega bo‘lmadi. Ahamiyatli jihati shundaki, emlangan guruhlar (G3 va G4) doza bog‘liq neytrallash tendensiyalarini namoyish etdi, bunda ayollarda neytrallash samaradorligi odatda yuqoriroq bo‘ldi. Qizig‘i shundaki, G3 ayol guruhi 42 va 112-kunlarda G4 guruhiga qaraganda yuqori neytrallash foiziga ega bo‘ldi, bu esa pastroq doza ham kuchli va uzoq muddatli immun javobni shakllantirish uchun yetarli bo‘lishi mumkinligini ko‘rsatdi.



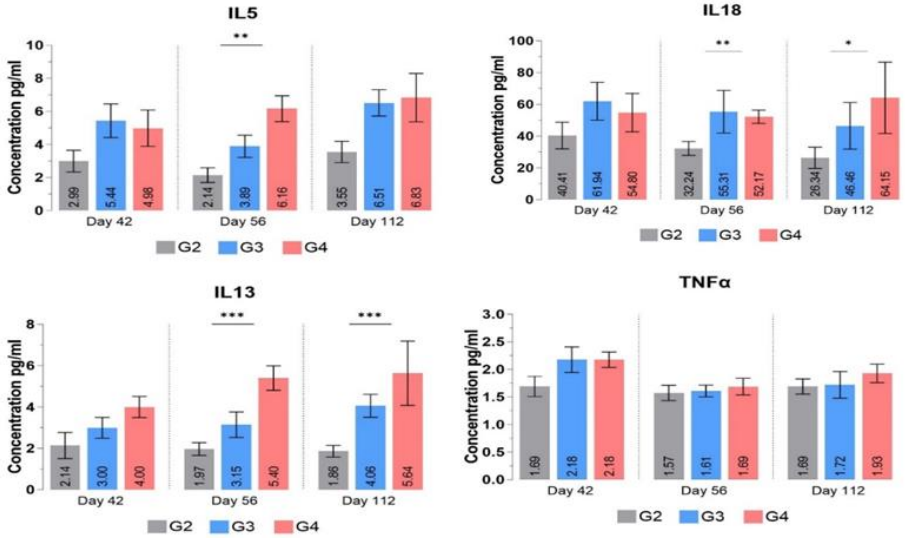
6-rasm. Erkak va urg‘ochi sichqonlarning SARS-CoV-2 surrogat virusini neytrallash samaradorligi. P-qiymatlar bir yo‘nalishli ANOVA yordamida tahlil qilindi (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

RENOVAC vaksinasining ta'siri natijasida hujayraviy immun javobi sezilarli o‘zgarishlarga uchradi (7-rasm). Xususan, CD3+ T-hujayralari ko‘rsatkichlari taloqda oshdi, lekin limfa tugunlarida dastlab kamayib, 112-kunga kelib barcha guruhlarda barqarorlashdi. CD4+ yordamchi T-hujayralari vaksina bilan emlangan guruhlarda, ayniqsa limfa tugunlarida, kechikkan, ammo kuchliroq javobni namoyon etdi. CD8+ sitotoksik T-hujayralar esa o‘rtacha darajada oshdi. Ushbu natijalar vaksinaning barqaror hujayraviy immun javobini shakllantirish qobiliyatini ko‘rsatadi.



7-rasm. Emlashdan so‘ng limfa tugunlari va taloqdagi CD hujayralari foizi. P-qiymatlari bir yo‘nalishli ANOVA yordamida tahlil qilingan (*p < 0.05).

Sitokin tahlili, aksariyat yallig‘lanish sitokinlari normal diapazonda qolgani va bu esa RENOVAC vaksinasining sitokin bo‘ronini qo‘zg‘atmaganligini ko’rsatdi. IL-2, IL-4 va IL-6 kabi ba’zi sitokinlar ko‘plab namunalarida aniqlanmagan bo‘lsada, IL-18 darajasi emlangan guruhlarda oshib, Th1 hujayralarining javobini rag‘batlantirishi mumkinligini anglatadi (8-rasm). Bundan tashqari, IL-5 va IL-13 sezilarli darajada oshib, Th2 vositachiligidagi antitana javoblarini kuchaytirganligini ko‘rsatdi. Kuzatilgan sitokin profili samarali immunomodulyatsiyani tasdiqlaydi va vaksina bilan qo‘zg‘atilgan immunitetda Th1 va Th2 yo‘llarining muhim rol o‘ynashini qo‘llab-quvvatlaydi. TNF-alfa darajalari esa ortiqcha immun javobning oldini oluvchi nazorat mexanizmini ko‘rsatmoqda.



8-rasm. Immunizatsiyadan so‘ng sitokinlarning konsentratsiyasi. P-qiymatlar bir yo‘nalishli ANOVA yordamida tahlil qilingan (*p < 0.05; **p < 0.01; ***p < 0.001).

Shunday qilib, dimer RBD - RENOVAC vaksinasi asosan T va B hujayralarini faollashtirib, Th2 subpopulyatsiyalarini hosil bo’lishini rag’batlantirishi, gumoral immunitetni shakllantirishi hamda CD8+ T hujayralarini faollashtirishi va TNF-α orqali immun javobni haddan tashqari kuchayishining oldini olishi mumkingli aniqlandi.

XULOSA

1. CHO hujayralarida SARS-CoV-2 RBD oqsili tadqiqotda sinalgan boshqa tojdor antigenlarga nisbatan ko‘proq miqdorda ekspressiyalanishi mumkin. Dimer RBD oqsili CHO hujayralarida ekspressiyalangan ikki xil butun va parchalangan holda uchrab, butun dimer RBD oqsili molekular massasi 50-55 kDa atrofida bo‘lib, ikki bosqichli suyuqlik xromatografiyasiga asoslangan protokol asosida 99%gacha tozalab olish mumkin.

2. Bioinformatik analizlar olingan dimer RBD antigeni tarkibida 22 ta MHCI va 7 ta MHCII T hujayra epitoplari, 18 ta chiziqli B hujayra epitopi (shulardan 10 tasi ilgari aniqlangan eskperimental epitoplar bilan mos) va 416-506 amino kislotalar oralig‘ida eksperimetal aniqlangan kuchlik B hujayra konformatsion epitoplari borligini va ularning hujayralarga muvaffaqiyatli taqdim bo‘la olishini ko‘rsatdi.

3. Spraguye Dawley kalamushlarida o‘tkazilgan preklinik toksikologik baholash natijasida RENOVAC mushak ichiga yuborilganda 25 µg/gacha bo‘lgan dozalarda yaxshi qabul qilnishi mumkinligini ko‘rsatdi. Taloq va timusda immun reaksiyaga xos o‘zgarishlar kuzatilishi mumkin. Vaksinatsiya natijasida sodir bo‘lgan o‘zgarishlarning ba’zilari statistik ahamiyatga ega bo‘lgan bo‘lsada, bu o‘zgarishlar laboratoriya kalamushlari uchun xabar qilingan tarixiy diapazonda bo‘lishi mumkin. Shuningdek, katta dozada ham gematologik, biokimyoviy yoki gistopatologik jihatdan toksikologik ahamiyatga ega bo‘lgan hech qanday o‘zgarishlar kuzatilmasligi aniqlanib va RENOVAC uchun hech qanday nojo‘ya ta’sir kuzatilmagan daraja (NOAEL) 25 µg/doza deb belgilandi.

4. Balb/c sichqonlarida RENOVAC ichki qorin bo‘shlig‘iga yuborilganda yuqori immunogen potensial ko‘rsatdi. 10 µg doza anti-RBD IgG va neytrallovchi antitanachalarni hosil qilish uchun yetarli bo‘lib, ularning darajasi uzoq muddat davomida barqaror saqlanib qolishi mumkin. Immunologik testlar vaksinaning asosan CD4+ yordamchi T hujayralarini rag‘batlantirganini va Th2 subtipiga differensiallanishini, shuningdek, gumoral immunitet uchun mas’ul bo‘lgan B hujayralarini faollashtirishini ko‘rsatdi. Bundan tashqari, CD8+ T hujayralari va IL-18 markerlaridagi o‘zgarishlar hujayraviy immunitetning faollashish ehtimolini ko‘rsatib, RENOVAC ning kengroq immunologik ta’siriga ega ekanligini hamda uzoq muddatli immunitetni rag‘batlantirish, organizmni viruslardan xoli bo‘lishiga ko‘maklashish qobilyati mavjudligini bildiradi.

SCIENTIFIC COUNCIL DSC.03/30.07.2024.B.179.01 FOR AWARDING SCIENTIFIC DEGREES OF THE CENTER OF ADVANCED TECHNOLOGIES

CENTER FOR ADVANCED TECHNOLOGIES

MUMINOV MUZAFFAR ISLOMJONOVICH

DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST SARS-COV-2 VIRUS

03.00.03-Molecular biology. Molecular genetics. Molecular biotechnology

ABSTRACT
OF THE DISSERTATION OF THE DOCTOR OF PHILOSOPHY (PHD) IN BIOLOGICAL SCIENCES

Tashkent-2025

This dissertation of PhD has been registered with the number B2024.3.PhD/B1238 at the Supreme Attestation Commission of the Ministry of Higher Education, Science and Innovation of the Republic of Uzbekistan.

The dissertation was completed at the Advanced Technologies Center under the Ministry of Higher Education, Science and Innovation.

The abstract of the dissertation is posted in three (Uzbek, English and Russian (resume)) languages on the on the website of the Scientific Council cat-dscphd.uz and on the "Ziyonet" information and educational portal (www.ziyonet.uz).

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Leading organisation:	Institute of the Chemistry of Plant Substances

The defense of the dissertation will be held at the meeting of the academic council number DSs.03/07.2024.B.179.01 at the Center for Advanced Technologies under the Ministry of Higher Education, Science and Innovation "13" august 2025 at 10^{00} (Address: 100174, Tashkent city, Almazor district, Talabalar shaharchasi street, 3A, Tel.: (99871) 227 43 21

The dissertation can be reviewed at the Information Resource Center of the Center for Advanced Technologies under the Ministry of Higher Education, Science, and Innovation (registered under №.4). Address: 100174, Tashkent, Almazar district, University Street, House 3A. Tel.: (+99871) 227 43 21, E-mail: catscience@exat.uz.

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INTRODUCTION (PhD dissertation abstract)

Importance and demand for the research. In the world, special attention is being paid to the development of effective and safe vaccines to prevent COVID-19. The goal of a vaccine is to stimulate an immune response by producing a foreign antigen that the immune system can recognize and respond to. Additionally, modern methods of vaccine development based on mRNA, viral vectors, protein subunits, and virus-like particles have been extensively analyzed, alongside traditional methods such as live attenuated vaccines. It is worth noting that each platform possesses unique mechanisms, as well as advantages and challenges, in global SARS-CoV-2 immunization. Protein subunit and virus-like particle vaccines offer several advantages: they are safe, non-infectious, do not cause disease, and are suitable for immunocompromised individuals. Therefore, the development of a local recombinant protein vaccine against COVID-19 caused by the SARS-CoV-2 virus holds great significance.

Globally, scientific research is underway on the development and implementation of recombinant protein vaccines, along with mRNA and vector vaccines, against the SARS-CoV-2 virus. In this regard, the expression of SARS-CoV-2 antigens, purification of RBD proteins using liquid chromatography methods, preclinical safety analysis of the vaccine in laboratory settings, determination of its effects on the immune system, formation of antibodies, cytokine levels, and cellular immune response are being studied. Such parameters are essential to minimize any serious adverse effects before entering clinical trials and to refine specific approaches in vaccine development. Hence, the development and analysis of a local recombinant protein vaccine against SARS-CoV-2 is necessary.

In our republic, certain achievements have been made in preventing infectious diseases, early detection and treatment of SARS-CoV-2 coronavirus, ensuring the availability of essential medicines for patients, developing import-substituting pharmaceuticals, and creating local recombinant protein vaccines. The President of the Republic of Uzbekistan, in the New Uzbekistan Development Strategy[1] for 2022–2026, set forth goals such as tripling the volume of pharmaceutical products, meeting 80% of domestic market demand, optimizing the volume and composition of imported products, and supporting local manufacturers. In implementing these goals, identifying the expression of SARS-CoV-2 antigens and developing and applying the RENOVAC vaccine based on the recombinant dimeric form of the RBD fragment of the SARS-CoV-2 virus is of critical importance.

This dissertation research serves, to some extent, the implementation of the tasks outlined in the following legal documents: the Presidential Decree of January 28, 2022, No. PF-60 “On the Development Strategy of New Uzbekistan for 2022–2026”; the Presidential Decree of July 25, 2020, No. PF-6035 “On Measures to

[1] The Decree of the President of the Republic of Uzbekistan No. PF-60 dated January 28, 2022 “On the Development Strategy of New Uzbekistan for 2022-2026

Mitigate the Coronavirus Pandemic, Improve Sanitary-Epidemiological Safety and Protect Public Health"; the Presidential Resolution of March 26, 2020, No. PQ-4649 "On Additional Measures to Prevent the Widespread of Coronavirus Infection in the Republic of Uzbekistan"; and the Presidential Decree of January 23, 2024, No. PF-20 "On Additional Measures to Regulate the Pharmaceutical Sector," along with other relevant normative and legal documents.

Correspondence of the research to the priority areas of development of science and technology of the Republic. This research was carried out in accordance with the priority direction of the development of science and technology of the republic - VI "Medicine and Pharmacology".

The degree of study of the problem. The world scientific literature provides information on the morphological structure, genetic size, and classification of coronaviruses, as well as on the characteristics of infectious diseases caused by viruses in various organisms (Woo et al., 2010; Cherry et al., 2017). Scientific studies have shown that some types of viruses belonging to the coronavirus family cause mild cold symptoms in humans, while others cause severe clinical conditions, including diseases accompanied by respiratory syndromes, and that coronaviruses cause infectious diseases such as diarrhea in cows and pigs, and hepatitis and encephalomyelitis in mice (Cherry et al., 2017; Fan et al., 2019). In the past two decades, two zoonotic coronaviruses, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), have been identified as causing epidemics in humans, killing thousands of people (Chan-Yeung and Xu, 2003; Zumla et al., 2015).

The widespread spread of COVID-19 has been driven by the discovery of the virus (Zhou et al., 2020), the complete genome sequence (Fan et al., 2020), the mechanisms of infection (Jackson et al., 2022; Khreefa et al., 2023), and the structure of the virus and its proteins (Huang et al., 2020; Yan et al., 2022). These studies have paved the way for targeted treatment of SARS-CoV-2, paving the way for diagnostic kits (Alhamid et al., 2022; El-Daly, 2024) and vaccine development. The development of vaccines based on different platforms to prevent the spread of COVID-19 has been reviewed in a number of literatures (Zhang et al., 2022; Rahman et al., 2022). Information on vaccines developed against COVID-19 and their clinical trials has been cited by the WHO (WHO COVID-19 vaccine tracking, 2025). It has been suggested that all available vaccines target the surface protein and its fragments of SARS-CoV-2 as antigens, with its ACE-2 receptor binding domain (RBD) being considered the most promising candidate (Sheikhshahrokh et al., 2020; Dai and Gao, 2021). In particular, mRNA vaccines produced by BioNTech/Pfizer and Moderna are based on the synthesis of the virus's crown protein after administration to the body (Polack et al., 2020; Baden et al., 2021), the ZF-2001 vaccine, which has undergone phase III clinical trials in our country and has shown 84.8% efficacy, was developed based on the RBD antigen of the coronavirus crown protein (Dai et al., 2020; Turdikulova et al., 2022), a local diagnostic test system for

detecting the SARS-CoV-2 virus was created by scientists of our republic, in particular by employees of the Center for Advanced Technologies (Sh.U.Turdikulova et al., 2022; Sh.N.Ibragimova, 2025), the full genome of the SARS-CoV-2 virus was sequenced by the Center for Advanced Technologies (A.Abdullaev et al., 2022; G.Esonova et al., 2022). et al., 2024) and the Center for Genomics and Bioinformatics (M.S.Ayubov et al., 2022; M.S.Ayubov et al., 2024) were sequenced and the trends in the spread of mutant variants in our country were analyzed. The domestic TOMAVAC vaccine, which can be consumed from tomato fruits, was developed based on a fragment of the crown protein of the virus (Z.T.Buriev et al., 2024). It is worth noting that this is the first time that a domestic recombinant protein vaccine against SARS-CoV-2 virus in CHO cells and its preclinical safety and immune profile have been developed.

The relevance of the research to the scientific research plans of the higher education or research institution where the dissertation was completed. The dissertation research was carried out within the framework of the target project of the Center for Advanced Technologies research plan No. M-2021-1 on the topic "Development of anti-SARS-CoV-2 DNA and recombinant protein vaccines using CHO animal cells" (2021-2023).

The purpose of the study is to develop a local recombinant protein vaccine against the SARS-CoV-2 virus in CHO cells and determine its preclinical safety and immune profile.

The objectives of the study:

Development of a protein subunit vaccine candidate against SARS-CoV-2 virus infection;

predicting T and B lymphocyte epitopes in a vaccine candidate using in silico bioinformatic tools;

assessment of the preclinical safety of the vaccine candidate in Sprague Dawley rats;

determination of the preclinical immune profile of the vaccine candidate in Balb/c mice.

The objects of the study were antigen synthesizing constructs, expressed antigens, CHO cells, Sprague Dawley rats, and Balb/c mice.

The subject of the study was the design and creation of vaccine antigens, the evaluation of their expression in CHO cells, and the determination of the safety and immunogenicity of the prepared vaccine candidate in laboratory animals.

Research methods. Molecular biology, biotechnology, bioinformatic software, biochemistry, microbiology, and statistical analysis methods were used during the research process.

The scientific novelty of the study is as follows:

The expression of several SARS-CoV-2 antigens in CHO cells was studied, and it was found that dimeric RBD protein is expressed in higher quantities compared to the tested spike proteins, with a yield of 20–50 mg/L after purification,

a molecular weight of approximately 50–55 kDa, identified in both intact and degraded forms when expressed in CHO cells; and using liquid chromatography methods, 99% purity of the intact form of the RBD protein was achieved and a purification protocol was developed;

For the first time, based on recombinant dimeric RBD protein obtained in CHO cells via genetic engineering, two concentrations of RENOVAC vaccine (10 μg/dose and 25 μg/dose) were developed;

When 25 μg dose of RENOVAC vaccine was administered intramuscularly to Sprague Dawley rats, changes observed in the spleen and thymus were associated with an immunological response to the vaccine, with no toxicological adverse changes observed, and the No-Observed-Adverse-Effect Level (NOAEL) was substantiated to be at the 25 μg/dose level;

Intraperitoneal administration to Balb/c mice showed good immunogenic potential of the vaccine at both low and high doses, but the 10 μg/dose vaccine produced sufficient levels of anti-RBD and virus-neutralizing antibodies that were found to persist in large amounts for a longer time;

For the first time in Uzbekistan, the mechanism of action of the vaccine was identified, primarily through activation of CD4+ helper T cells leading to an immune response, and this T-cell activation subsequently mediated the formation of humoral immunity through activation of B cells, and it was found that changes in CD8+ cells and indicators such as interleukin-18 may activate cellular immunity after vaccination.

The practical results of the study are as follows:

The RENOVAC vaccine, based on a recombinant dimer form of the RBD fragment of the SARS-CoV-2 virus, was developed and produced at the CAT-Biotech experimental testing complex;

The dimeric RBD protein was formulated with aluminum hydroxide and safety and immunogenicity studies were conducted in rats and mice at two different doses;

Preclinical studies of the RENOVAC vaccine revealed its safety and high protection, as well as the scientific basis for the mechanism of action.

Reliability of the study results is evidenced by the use of modern molecular biology, bioinformatics, biochemistry and biotechnology methods, preclinical studies in accordance with the requirements of good laboratory practice (GLP), with the permission of the ethics committee of the relevant institutions, audit of the results, and the determination of differences between the test and placebo groups using the one-way ANOVA statistical method, and the determination of differences between the placebo and control groups using the t-test analysis.

Scientific and practical significance of the research results. The scientific significance of the research results lies in the ability of the low dose of the RBD-RENOVAC dimer vaccine to induce a high level of target-specific IgG and provide long-term immunity with significant neutralization efficiency, the creation of a

platform for the development of recombinant protein vaccines using mammalian cells, and the high efficacy of the dimeric RBD protein not only facilitating its production but also increasing its potential as a vital component of an expanded vaccine platform, serving in the development of recombinant protein-based vaccines against other emerging infectious diseases.

The practical significance of the research results is that the RENOVAC protein subunit vaccine based on the dimer RBD construct was developed and its safety and immune profile recommended it as an effective vaccine candidate for the prevention of SARS-CoV-2. This platform in Uzbekistan will serve in the development of other vaccines as well as in increasing preparedness for infectious diseases, future pandemics, and biological threats.

Implementation of the research results. Based on the scientific results obtained during the development and implementation of the recombinant protein vaccine against the SARS-CoV-2 virus:

A recombinant protein vaccine candidate against the SARS-CoV-2 virus was produced in a small batch at the CAT-Biotech experimental pilot production complex (according to the certificate No. 5/68 dated March 17, 2025, from the Ministry of Higher Education, Science and Innovation of the Republic of Uzbekistan). As a result, the technology for purifying proteins through liquid chromatography developed during the vaccine creation process was established;

The soluble recombinant S protein cells produced in CHO cells were registered by the World Health Organization (WHO) on the COVID-19 vaccine development platform (https://www.who.int/teams/blueprint/covid-19/covid-19-vaccine-tracker-and-landscape). As a result, it enabled the development and comparison of other vaccines;

The DNA sequence of the dimer RBD protein against the SARS-CoV-2 coronavirus has been registered in the GenBank and National Center for Biotechnology Information (NCBI) databases under the accession numbers PV232931 and XPR33336.1 (https://www.ncbi.nlm.nih.gov/nuccore/PV232931.1/). As a result, it allowed the improvement and comparative analysis of DNA or protein sequences for the development of new vaccines.

Testing of the research results. The results of this dissertation research were presented at 2 international and 4 republican scientific and practical conferences.

Publication of the research results. 7 scientific works on the topic of the dissertation, including 3 scientific articles, were published in prestigious scientific journals recommended by the Supreme Attestation Commission of the Republic for publishing scientific results of dissertations, including 1 in republican publications and 2 in foreign journals.

Structure and volume of the dissertation. The dissertation work consists of an introduction, 3 chapters, a conclusion, a list of references and appendices. The volume of the dissertation is 97 pages.

MAIN CONTENT OF THE DISSERTATION

The introduction justifies the relevance and necessity of the topic, defines the goals and objectives of the research, describes its object and subject. Also, the correspondence of the research to the priority areas of development of science and technology of the republic is indicated. The scientific novelty and practical results of the work are described, and the scientific and practical significance of the results obtained is highlighted. The prospects for the implementation of the research results into practice, information on published scientific works and the structure of the dissertation are provided.

The first chapter of the dissertation, titled **"COVID-19 pandemic and development of vaccines to prevent the infection"** analyzes the impact of the COVID-19 pandemic on the world and Uzbekistan, the structure, evolution, and pathogenesis of the virus, as well as the history of vaccines and the significance of vaccination programs. This chapter provides an in-depth examination of vaccine platforms used to control the COVID-19 pandemic, particularly whole-organism vaccines and recombinant DNA (rDNA)-based vaccine platforms. Additionally, it discusses the unique characteristics of candidate vaccines, highlighting the advantages and limitations of different platforms. At the end of the chapter, an overview of vaccine development stages and the accelerated processes used during the COVID-19 pandemic is provided.

The second chapter of the dissertation, titled **"Materials and methods used in vaccine development and preclinical assessment"** outlines the stages of the research, the materials, and the methods applied during the study. This chapter is structured into three major sections. The first section describes the research methods used in recombinant protein vaccine development, including protein antigen design, expression vector creation, vector preparation, antigen expression in CHO cells, bioinformatic analysis of epitopes in the dimer RBD protein, and purification methods using liquid chromatography. Routine analytical protocols used in these processes are also mentioned. The second and third sections of the chapter focus on preclinical safety and immune profile studies, respectively. They comprehensively describe the ethical aspects of laboratory animal research, the study design, and the protocols used to assess predefined parameters. Finally, the chapter concludes with details on statistical programs and analysis methods used for group comparisons, specifically explaining how ANOVA and t-tests were conducted.

In the third chapter of the dissertation, titled **"Results and discussion of vaccine development and preclinical assessment"** the first section presents and analyzes the results of the vaccine development process in accordance with the research objectives. The second section provides the results and analysis of studies conducted to assess the safety profile of the vaccine in Sprague Dawley rats. Finally, the third section presents and analyzes the results of the immunoprofile analysis conducted in Balb/c mice.

During the study, 4 vaccine antigens were designed based on the SARS-CoV-2 crown protein and its RBD fragment and cloned into the pcDNA3.4 plasmid under the CMV promoter using the XbaI and AflII restriction enzymes (Figure 1A).

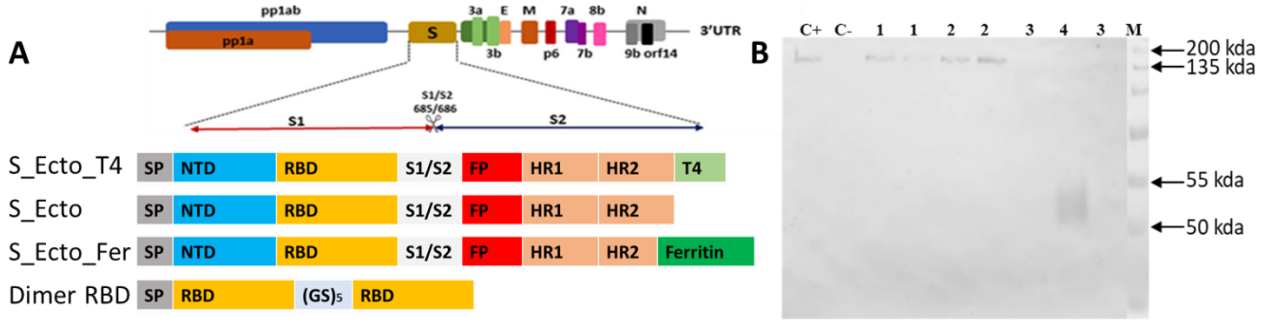

Figure 1. Created antigen constructs and their expression analysis. A – structure of antigens; B – expression analysis of antigens in CHO cells: 1 – S_Ecto_T4, 2 – S_Ecto, 3 – S_Ecto_Fer, 4 – Dimer RBD.

S_Ecto_T4: Encodes the spike ectodomain (amino acids 13-1213) and encodes a T4 foldon trimerization domain at the C-terminal end to facilitate stable trimer formation. **S_Ecto:** Similar to the previous construct but lacking the T4 foldon sequence, allowing comparison of the effect of the trimerization domain on expression and antigen stability. **S_Ecto_Fer:** Contains a truncated HR2 fragment and the *H.pylori* ferritin multimerization protein. Aims to enhance antigen presentation by forming multimers. **Dimer RBD:** This construct encodes a tandemly repeated RBD sequence linked to a 5xGS linker to increase antigen density and enhance immune recognition, and a modified version of this construct additionally encodes a 6-histidine tag at the C-terminal end of the target protein to facilitate purification.

Numerous studies have shown that efficient expression of the SARS-CoV-2 crown protein and its fragments using recombinant technologies is difficult. Therefore, in an initial study, the expression of the constructed constructs in CHO cells was evaluated and it was found that the dimeric RBD protein was expressed more efficiently than the other proteins (Figure 1B).

Due to the high expression of the dimeric RBD protein compared to other constructs, this construct was selected for vaccine development, and subsequent studies using bioinformatic programs predicted MHCI/II T cell epitopes in the candidate antigen, and the RLFRKSNLK MHCI epitope in the protein was shown to not only be compatible with different HLA alleles, but also to be confirmed in empirical studies and to effectively activate T cells in experimental assays. These types of epitopes are essential for inducing cytotoxic CD8+ T cell responses and targeting infected cells. MHCII epitopes, on the other hand, activate CD4+ helper T cells that support antibody production and coordinate broader immune responses. Epitopes such as FERDISTEI and FASVYAWNR in the dimeric RBD were found to have strong binding potential to multiple HLA alleles.

The 10 predicted lineage B cell epitopes within the dimer RBD were found to have a high degree of similarity to previously confirmed epitopes in the literature (Table 1).

Table 1 . Confirmed B cell linear epitopes with suitable coming prophecy Dimer RBD epitopes .

No.	Start	Stop	Predicted epitope amino acid sequence	Spike protein position
1.	26	45	ATRFASVYAWNRKRISNCVA	344-363
2.	55	60	SFSTFC	373-378
3.	86	109	GDEVRQIAPGQTGKIADYNYKLPD	404-427
4.	122	188	NLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQ AGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQ	440-506
5.	201	216	HAPATVCGPKKSTNLV	519-534
6.	258	276	NATRFASVYAWNRKRISNC	343-361
7.	318	342	RGDEVRQIAPGQTGKIADYNYKLPD	403-427
8.	355	382	NLDSKVGGNYNYLYRLFRKSNLKPFERD	440-467
9.	384	420	STEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGV GY	469-504
10.	434	452	HAPATVCGPKKSTNLVKNK	519-537

Bioinformatic analysis of conformational B cell epitopes confirmed the presence of several epitopes in the dimer RBD protein that correspond to previously identified regions of immunological relevance (Figure 2). Of these identified epitopes, residues 416–469 are a segment critical for ACE2 receptor binding, including important residues such as K417. The epitope between 441–463 contains a key loop critical for ACE2 interaction. Finally, the epitope between 495–506 is located around N501, a residue critical for receptor binding and receptor binding.

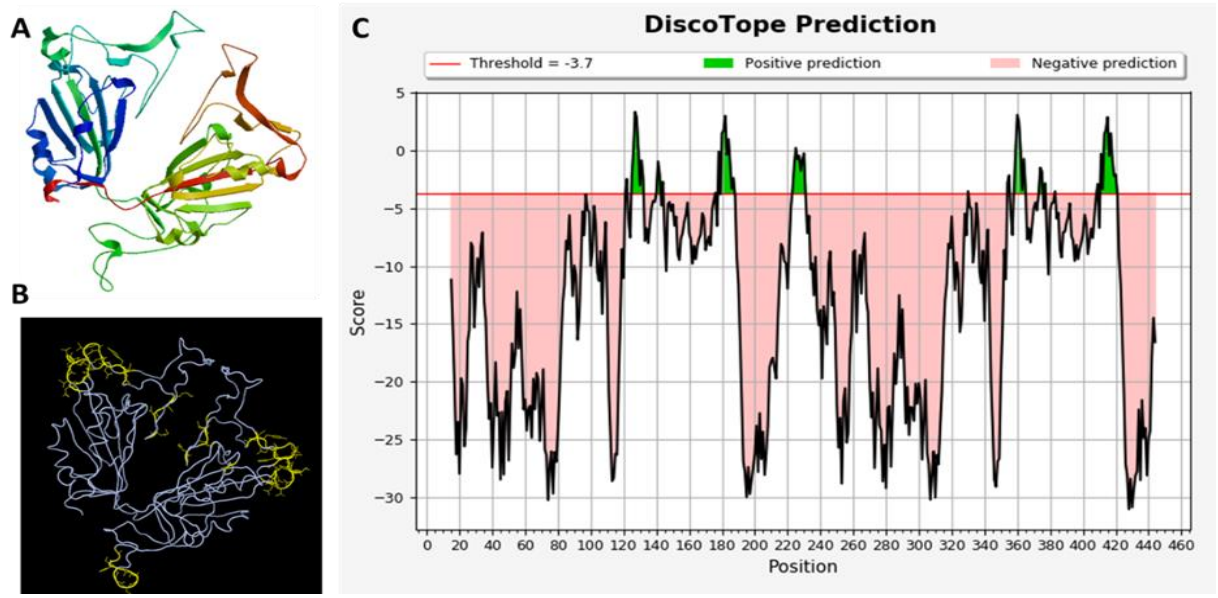

Figure 2. Predicted 3D structure and conformational B cell epitopes of the dimeric RBD vaccine candidate. (A) Predicted 3D model of the dimeric RBD protein. (B) Visualization of predicted conformational B cell epitopes in the dimeric RBD vaccine as a 3D model. (C) Predicted conformational B cell epitopes in the dimeric RBD candidate.

When expressed in CHO cells, the dimeric RBD was found to exist in both intact and cleaved forms (Figure 3A). The molecular mass of the intact dimeric RBD protein was ~50–55 kDa, as expected, and it is likely that the cleaved form was formed by enzymatic cleavage of the monomeric RBD residues.

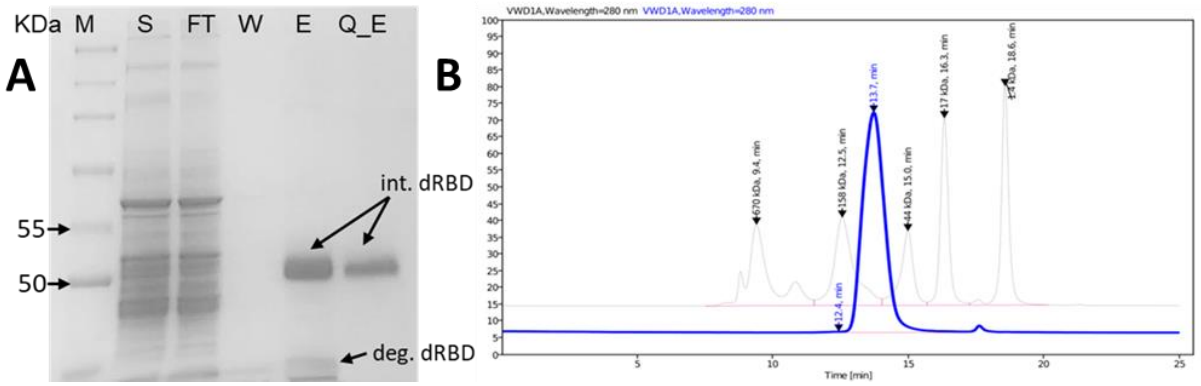

Figure 3. Purification of the dimer RBD protein and determination of its purity. A. PAAG analysis of purified dimer RBD fractions; S - initial culture fluid; FT - protein fraction not bound to the affinity sorbent; W - washing of the fraction with a buffer containing 40 mM imidazole; E - elution of the target fraction with a buffer containing 250 mM imidazole; Q_E - elution of the target fraction with a buffer containing 250 mM imidazole; Q_E - elution of the target protein from the ion exchange column. C. SEC-HPLC analysis of the purified antigen.

The purity of the dimeric RBD purified by two-step nickel affinity and ion exchange liquid chromatography was confirmed to be at least 99% by SEC-HPLC analysis (Figure 3B). It was also determined that the amount of pure dimeric RBD was approximately 20-50 mg per liter of cell culture.

μg antigen per dose), and a high-dose (25 μg antigen per dose) were aseptically packaged using aluminum hydroxide (alum) as an adjuvant and developed under the name RENOVAC.

In accordance with the research objectives, preclinical studies were conducted, initially in Sprague Dawley rats, 3 types of test vaccines prepared in a total of 2 doses were injected with an interval of 7 days, and studies were conducted to determine the safety profile **of the vaccine with 14-day toxicity (G1 - placebo, G2 - low-dose vaccine and G3 - high-dose vaccine) and 14-day toxicity with a 28-day recovery period (G4R - placebo and G5R - high-dose vaccine)** .

During the study, no deaths or abnormal clinical signs were observed in rats administered placebo, low-dose, and high-dose RENOVAC vaccine, and routine assessments did not reveal any treatment-related abnormalities, confirming the safety of the vaccine and confirming that there were no clinically significant changes in appearance, health status, or behavior.

Male and female rats in all control and treatment groups showed normal and expected increases in body weight. This indicates normal growth and development of the animals and suggests that RENOVAC vaccine had no adverse effects on general health. However, a slight dose-related decrease in body weight was observed at day 14 for the first three groups and at day 42 in the recovery groups. These changes were not statistically significant and therefore indicate that this condition is not related to vaccination.

Hematological studies showed clear but non-toxic changes in baseline parameters, reflecting immunological responses rather than adverse effects (Table 2). For example, males (particularly in the G3 group) showed a dose-related increase in white blood cell (WBC) counts at day 15 and a slight decrease at day 43, and only females in the G2 group showed a statistically significant increase in mean corpuscular hemoglobin (MCH) at day 15. Minimal, non-dose-related changes in hemoglobin (HGB), hematocrit (HCT), and platelet count (PLT) also demonstrated physiological variability and confirmed the absence of vaccine-related toxicity.

Table 2. RENOVAC vaccine next in rats hematology parameters

Parameters		**Male**					**Female**				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
WBC (103 / µL)	Mean	15.97	16.13	19.02	13.68	10.08	12.63	12.43	12.50	7.07	8.12
	SD	3.72	6.43	5.06	3.80	3.09	4.16	4.16	5.67	1.78	1.06
HGB (g/ dL)	Mean	12.65	13.07	12.62	13.38	13.92	12.05	12.50	12.55	12.52	12.92
	SD	0.52	0.45	0.52	0.97	1.08	0.61	0.50	0.82	0.74	0.36
HCT (%)	Mean	39.68	40.35	39.27	42.90	44.95	35.55	36.17	37.12	39.13	39.33
	SD	1.61	2.02	1.83	3.11	3.83	1.82	1.32	2.36	2.43	0.96
MCH (pg)	Mean	17.23	17.95	17.28	16.53	16.28	18.25	18.97 *	18.00	17.03	17.53
	SD	0.78	0.92	0.71	0.60	0.69	0.40	0.39	0.67	0.52	0.50
PLT (103 / µL)	Mean	1067.3	969.3	999.1	801.0	944.3	1023.6	978.0	957.3	870.1	858.5
	SD	156.36	103.71	156.21	160.22	123.38	121.65	116.44	181.60	86.62	120.65

Note : N = 6; Mean = average value ; SD = Standard deviation G1-G3 day 15, G4R-G5R day 43.
Key : * = Group average placebo at p value <0.05 control from the group noticeable at the level difference does .

On day 15, differential leukocyte counts showed a dose-dependent statistically significant increase in neutrophils and a relative decrease in lymphocyte counts in males in the G3 group (Table 3), whereas leukocyte counts in females and males in the primary recovery group were not significantly different compared with the respective control groups; these hematological changes, especially the initial increase in WBC and neutrophils, are consistent with the expected innate immune response after vaccination, as neutrophils play an important role in early protection against pathogens and in the development of adaptive immunity. The transient decrease in the lymphocyte percentage, however, was associated with the initial neutrophil increase and reflects the natural dynamic changes of the immune system during the early activation phase, confirming that these changes are a typical immune response and not a toxic or adverse effect of the vaccine.

Table 3. From the RENOVAC vaccine then in rats differential leukocytes number (%)

Parameters		Male					Female				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
Neutrophils	Mean	22.33	24.00	27.50 *	23.17	24.33	24.67	23.50	25.67	25.50	25.33
	SD	2.25	2.83	3.62	3.37	2.16	3.67	2.81	3.01	2.66	1.63
Lymphocytes	Mean	76.17	74.33	71.33 *	75.67	74.17	74.00	75.00	73.00	73.17	73.17
	SD	1.94	2.73	3.27	3.61	2.99	4.20	2.37	3.10	2.79	2.32

Note : N = 6; Mean = average value ; SD = Standard deviation G1-G3 day 15, G4R-G5R day 43.
Key : * = Group average placebo at p value <0.05 control from the group noticeable at the level difference does .

Clinical chemistry analyses performed after vaccination revealed dynamic, dose-dependent changes that indicated physiological and metabolic adaptations (Table 4). On day 15, a dose-dependent increase in glucose (GLU) levels was observed in both males and females. At the same time, alkaline phosphatase (ALP) levels in males and glutamate oxaloacetate transaminase (GOT) levels in females were significantly increased, indicating mild and transient liver stress. These changes are consistent with metabolic, hepatic, and renal adaptations that occur with routine vaccine exposure and are not toxicological effects.

Table 4. From the RENOVAC vaccine then in rats clinical chemistry assessment.

Parameters		Male					Female				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
GPT (U/L)	Mean	55.58	58.53	59.88	52.10	36.03*	53.23	59.12	51.88	49.37	43.72
	SD	13.29	7.35	10.67	10.41	5.10	10.38	9.93	7.52	9.10	8.09
GOT (U/L)	Mean	104.73	97.38	111.77	84.57	91.60	83.33	99.13	121.07*	93.15	108.17
	SD	14.69	26.71	21.05	8.00	12.21	11.98	17.58	39.14	12.69	24.16
ALP (U/L)	Mean	341.83	509.83*	453.67	388.17	313.67	251.83	262.17	328.67	220.50	239.67
	SD	26.83	148.68	100.77	106.15	70.60	65.24	73.20	54.77	93.13	71.01
BUL (mg / dL)	Mean	34.47	29.22*	34.12	27.55	36.05*	32.23	34.68	36.22	34.52	37.68
	SD	4.81	1.35	3.99	4.63	3.18	6.15	4.28	6.66	2.41	6.08
GLU (mg / dL)	Mean	97.10	107.78	115.25	142.62	115.28	101.52	104.13	113.83	91.87	111.92*
	SD	8.16	22.64	17.58	30.00	13.56	14.11	10.85	17.70	13.04	6.72
CHOLE (mg / dL)	Mean	60.50	65.50	64.33	53.83	53.00	71.00	71.67	66.50*	76.17	71.83
	SD	3.73	5.61	9.93	8.66	5.90	12.49	11.22	6.50	10.21	7.94
TRIG (mg / dL)	Mean	89.52	67.75	71.12	59.17	39.07*	56.87	50.98	45.95	33.45	29.53
	SD	19.77	12.65	26.33	13.56	15.78	19.36	22.28	15.21	8.32	9.18
ALB/GLO	Mean	0.68	0.67	0.64	0.62	0.68*	0.66	0.71	0.73	0.73	0.67
	SD	0.06	0.06	0.06	0.04	0.05	0.06	0.06	0.06	0.03	0.08
CBUN (mg / dL)	Mean	16.08	13.63*	15.92	12.86	16.82*	15.04	16.19	16.90	16.11	17.59
	SD	2.25	0.63	1.86	2.16	1.48	2.87	2.00	3.11	1.12	2.84
K (mmol /L)	Mean	4.68	4.65	4.68	4.43	4.66	4.32	4.39	4.55	4.48	3.91*
	SD	0.25	0.45	0.31	0.32	0.32	0.48	0.13	0.57	0.28	0.12
Cl (mmol /L)	Mean	100.88	105.28	100.27	101.77	102.05	105.18	101.55	101.47	103.13	100.77*
	SD	1.17	9.77	0.98	0.57	1.12	8.57	0.95	1.08	0.74	1.00

Note : N = 6; Mean = average value ; SD = Standard deviation G1-G3 day 15, G4R-G5R day 43.
Key : * = Group average placebo at p value <0.05 control from the group noticeable at the level difference does .

The decrease in cholesterol (CHOLE) levels in females may be related to changes in lipid metabolism during immune activation. By day 43, most clinical parameters had returned to baseline levels, reflecting a systemic recovery process. In particular, the decrease in triglyceride (TRIG) and alanine transaminase (GPT) levels in males indicates recovery of liver function, while the slight decrease in potassium (K) and chloride (Cl) levels in females indicates mild electrolyte changes that are not clinically significant. The increase in BUL and albumin-globulin (ALB) ratio in the recovery groups reflects the recovery of protein metabolism. Overall, these results confirm that the observed metabolic changes are part of the expected immunological response.

Urinalysis on day 15 showed a decrease in urine specific gravity and an increase in pH in both males and females, but these changes were only statistically significant

in females. During the recovery period, both parameters were consistent with those in the control groups. No significant differences were observed in all other urinalysis parameters and no histopathological correlation was found. A general pathological examination of the groups did not reveal any pathologically significant lesions compared to the control groups.

After surgical pathology examination, organs such as liver, kidneys, adrenal glands, testes/ovaries, thymus, spleen, brain, and heart were removed, weighed, and analyzed for absolute and relative weight changes, and the results showed transient and non-harmful changes such as spleen enlargement associated with the immune response, thymus weight changes associated with the T-cell response, and increased relative heart and brain weights in females (Table 5). Most of the changes had resolved by day 43 and were considered to be related to physiological processes as no histopathological changes were detected.

Table 5. RENOVAC vaccine from use then terminal body in rats to the weight relative organ weight

Organization/ groups		Male					Female				
		G1	**G2**	**G3**	**G4R**	**G5R**	**G1**	**G2**	**G3**	**G4R**	**G5R**
Liver	Mean	4.43	4.27	4.44	4.58	4.01 *	4.03	4.10	4.09	4.21	4.42
	SD	0.50	0.13	0.25	0.46	0.19	0.41	0.45	0.20	0.72	0.54
Heart	Mean	0.37	0.37	0.36	0.36	0.35	0.35	0.40 *	0.38	0.37	0.38
	SD	0.02	0.03	0.02	0.03	0.02	0.03	0.03	0.02	0.03	0.03
Thymus	Mean	0.12	0.10	0.11	0.10	0.11	0.15	0.14	0.16	0.13	0.18 *
	SD	0.03	0.03	0.03	0.03	0.02	0.05	0.02	0.05	0.02	0.03
Brain	Mean	0.79	0.72	0.76	0.72	0.67	0.88	0.95*	0.91	0.89	0.93
	SD	0.07	0.04	0.07	0.13	0.07	0.06	0.04	0.02	0.09	0.16

Note : N = 6; Mean = average value ; SD = Standard deviation G1-G3 day 15, G4R-G5R day 43.
Key : * = Group average placebo at p value <0.05 control from the group noticeable at the level difference does .

Histopathological analysis to the results because, no how in the organ treatment with related noticeable changes not detected, only in group G3 two male and one in a woman minimal follicular spleen hyperplasia was observed (Figure 4).

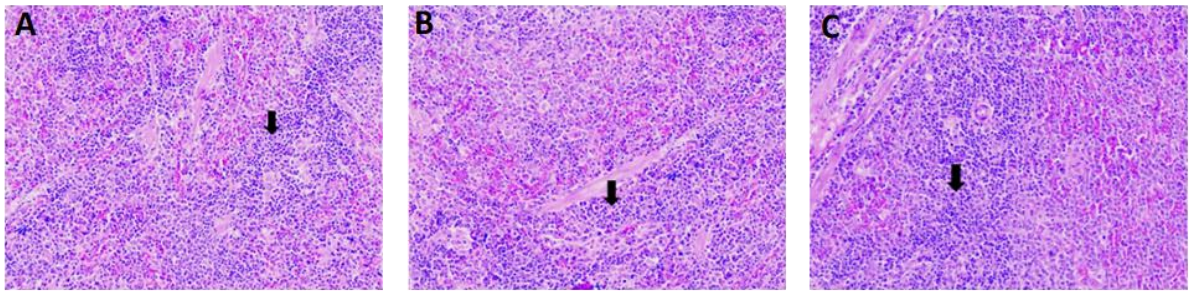

Figure 4. Formation of follicular cell hyperplasia (arrows). A and B - G3 males and C - G3 females.

Importantly, the spleens of the low-dose and recovery-phase groups were histologically similar when compared with the corresponding control groups, indicating that the observed changes were transient and dose-related.

In accordance with the final task of the study, preclinical studies were continued and **the immune profile of the vaccine** was studied in Balb/c mice. For this, the vaccine was injected intraperitoneally in 3 doses with an interval of 14 days, according to the groups, in phosphate buffered saline - PBS (G1), placebo (G2), low (G3) and high (G4) doses, and the formation of specific antibodies against the RBD protein, the formation of virus-neutralizing antibodies, and changes in the cellular and cytokine profile were studied at different periods.

The aluminum hydroxide-formulated dimer RBD vaccine induced high levels of specific IgG antibodies at all time points in both the low-dose (G3) and high-dose (G4) groups. The levels of anti-RBD IgG and neutralizing antibodies were almost undetectable in the phosphate buffer group, and since there were no statistically significant differences between the placebo and PBS control groups, the PBD group was removed from the graphs to ensure more accurate results. Anti-RBD IgG optical density (OD) values increased after each vaccine administration, reaching a maximum after the third dose (day 42) and then decreasing significantly until day 112 (Figure 5A).

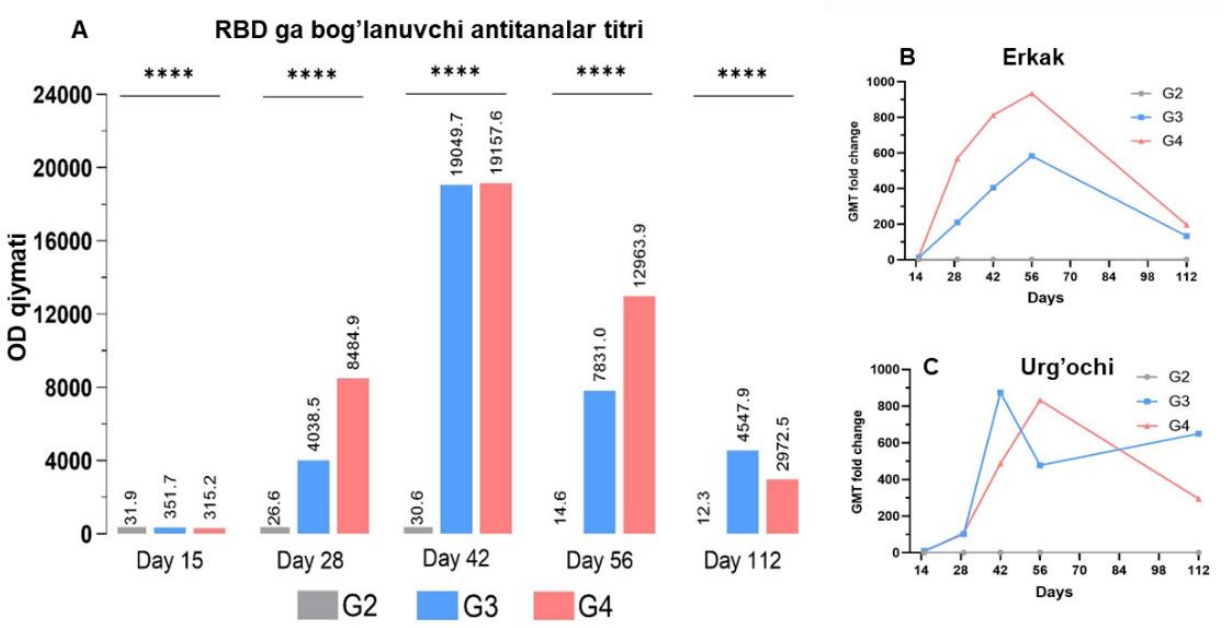

Figure 5. Changes in the GMT value of IgG binding to serological RBD in male and female mice. P-values were analyzed by one-way ANOVA (****p < 0.0001).

Geometric mean titer (GMT) analysis showed gender differences, with males increasing GMT proportionally to vaccine dose, while females responded more strongly to the 10 μg dose (G3). Females in the low-dose group maintained antibody titers for longer, indicating a stronger antibody response than males.

The results of the neutralization assay of the surrogate SARS-CoV-2 virus showed that the antibodies generated after vaccination could bind to the SARS-CoV-2 antigen conjugate and inhibit it to a high degree (Figure 6). At both doses, male

and female mice showed high neutralization activity, while the control groups did not have a significant inhibitory ability. Importantly, the vaccinated groups (G3 and G4) showed dose-dependent neutralization trends, with neutralization efficiency generally higher in females. Interestingly, the G3 female group had higher neutralization percentages than the G4 group at days 42 and 112, indicating that even a lower dose may be sufficient to induce a strong and long-lasting immune response.

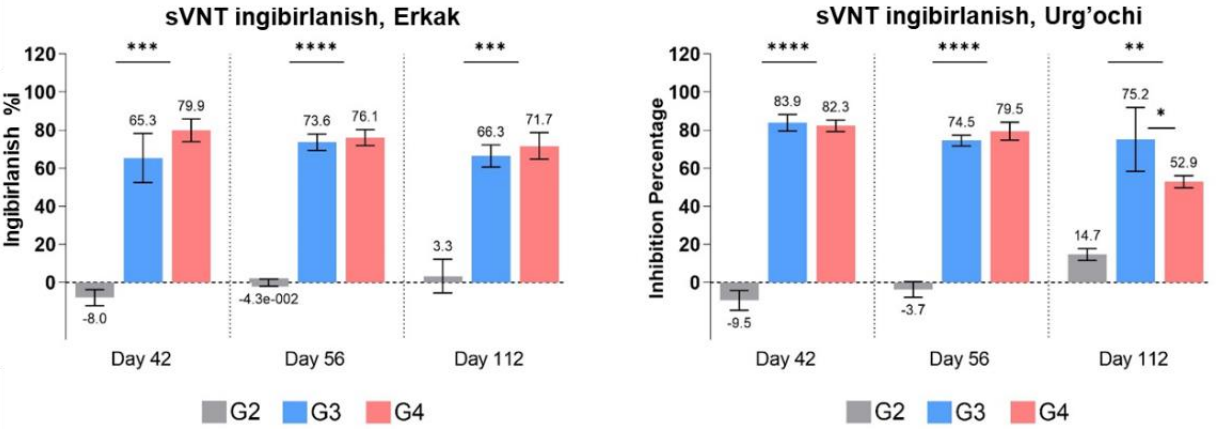

Figure 6. Male and accent SARS-CoV-2 surrogate in mice virus neutralization effectiveness. P- values one using one- way ANOVA analysis (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

The cellular immune response was significantly altered by RENOVAC (Figure 7). In particular, CD3+ T-cell counts increased in the spleen, but initially decreased in lymph nodes, and stabilized in all groups by day 112. CD4+ helper T-cells showed a delayed but stronger response in the vaccine-vaccinated groups, especially in lymph nodes. CD8+ cytotoxic T-cells, on the other hand, were moderately increased. These results demonstrate the ability of the vaccine to induce a sustained cellular immune response.

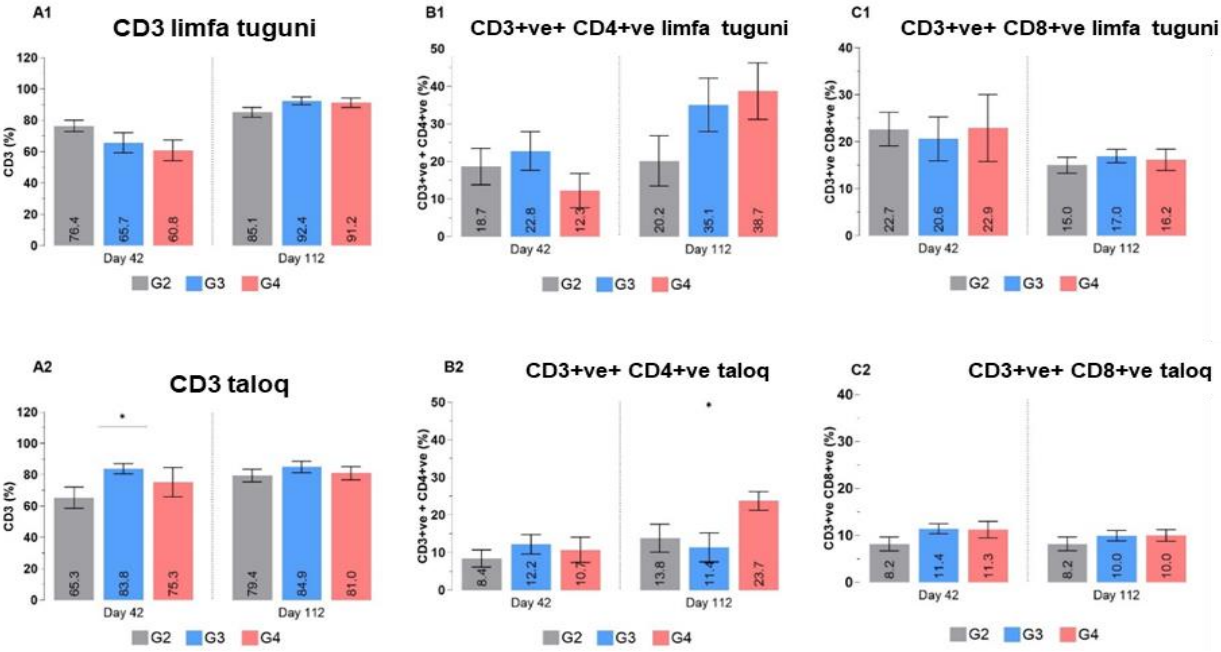

Figure 7. Percentage of CD4+ cells in lymph nodes and spleen after vaccination. P-values were analyzed using one-way ANOVA (*p < 0.05).

Cytokine analysis showed that most inflammatory cytokines remained within normal ranges, suggesting that RENOVAC vaccine did not induce a cytokine storm. Although some cytokines such as IL-2, IL-4, and IL-6 were not detected in most samples, IL-18 levels were increased in the vaccinated groups, suggesting that they may promote a Th1 cell response (Figure 8). In addition, IL-5 and IL-13 were significantly increased, suggesting that they enhanced Th2-mediated antibody responses. The observed cytokine profile confirms effective immunomodulation and supports the important role of Th1 and Th2 pathways in vaccine-induced immunity. TNF-alpha levels, on the other hand, indicate a control mechanism that prevents an excessive immune response.

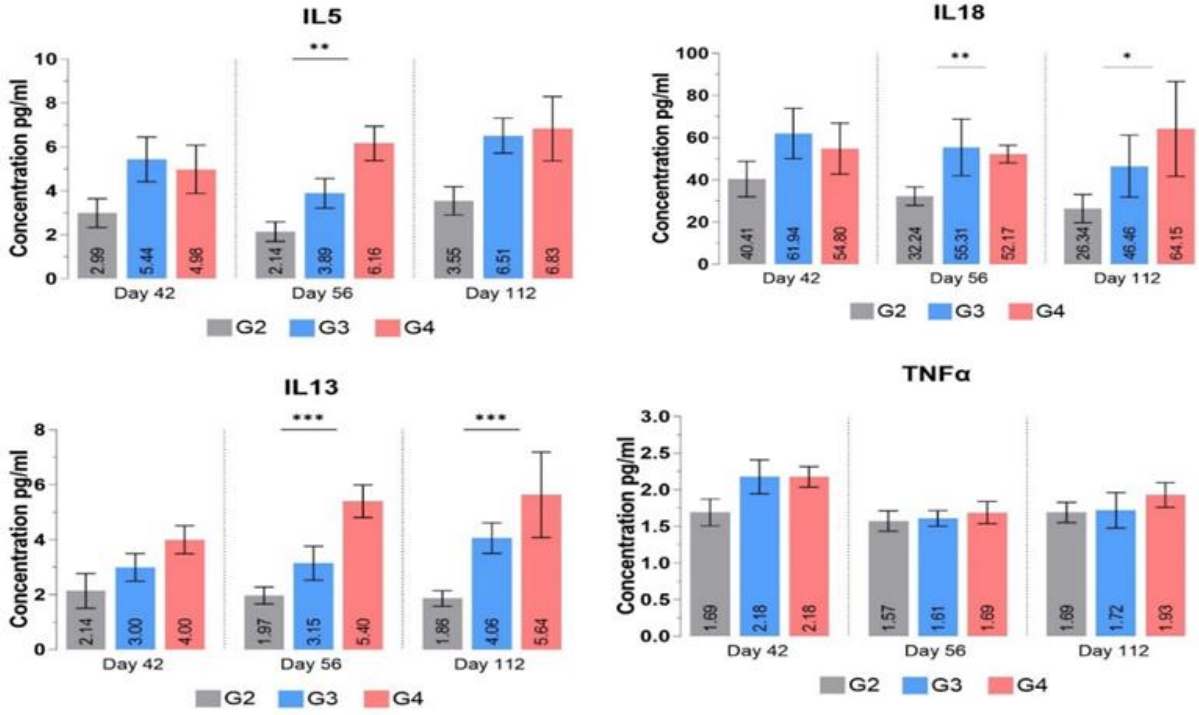

Figure 8. Cytokine concentrations after immunization. P-values were analyzed using one-way ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001).

Thus, it was found that the dimeric RBD - RENOVAC vaccine can mainly activate T and B cells, stimulate the formation of Th2 subpopulations, form humoral immunity, and activate CD8+ T cells and prevent excessive enhancement of the immune response through TNF-α.

CONCLUSION

1. The SARS-CoV-2 RBD protein in CHO cells can be expressed in greater amounts compared to other tested coronavirus antigens in the study. The dimer RBD protein expressed in CHO cells appears in both intact and fragmented forms, with the molecular mass of the intact dimer RBD protein being around 50–55 kDa. It can be purified up to 99% using a protocol based on two-step liquid chromatography.

2. Bioinformatic analyses showed that the obtained dimeric RBD antigen contains 22 MHC I and 7 MHC II T cell epitopes, 18 linear B cell epitopes (10 of

which match previously identified experimental epitopes), and experimentally identified strong B cell conformational epitopes in the amino acid range of 416–506, and that they can be successfully presented to cells.

3. The preclinical toxicological evaluation conducted in Sprague Dawley rats demonstrated that RENOVAC was well tolerated at doses up to 25 μg/animal when administered intramuscularly. Immunological response-related changes were observed in the spleen and thymus. Although some changes following vaccination were statistically significant, they remained within the historical range reported for laboratory rats. Furthermore, no hematological, biochemical, or histopathological toxicological abnormalities were detected even at high doses, leading to the determination of the No Observed Adverse Effect Level (NOAEL) for RENOVAC as 25 μg/dose.

4. RENOVAC exhibited a high immunogenic potential when administered intraperitoneally in Balb/c mice. A 10 μg dose was sufficient to induce significant levels of anti-RBD IgG and neutralizing antibodies, which remained stable for an extended period. Immunological assays revealed that the vaccine primarily stimulated CD4+ helper T cells, promoting their differentiation into the Th2 subtype and activating B cells responsible for humoral immunity. Additionally, changes in CD8+ T cells and IL-18 markers suggested a potential activation of cell-mediated immunity, indicating a broader immunological effect of RENOVAC and its ability to stimulate long-term immunity, potentially aiding in viral clearance.

НАУЧНЫЙ СОВЕТ DSC.03/30.07.2024.B.179.01 ПО Пприсуждению НАУЧНЫХ СТЕПЕНЕЙ ЦЕНТРА ПЕРЕДОВЫХ ТЕХНОЛОГИЙ

ЦЕНТР ПЕРЕДОВЫХ ТЕХНОЛОГИЙ

МУМИНОВ МУЗАФФАР ИСЛОМЖОНОВИЧ

РАЗРАБОТКА РЕКОМБИНАНТНОЙ БЕЛКОВОЙ ВАКЦИНЫ ПРОТИВ ВИРУСА SARS-COV-2.

03.00.03 – Молекулярная биология. Молекулярная генетика. Молекулярная биотехнология

АВТОРЕФЕРАТ
ДИССЕРТАЦИИ ДОКТОРА ФИЛОСОФИИ (PHD) ПО БИОЛОГИЧЕСКИМ НАУКАМ

Ташкент-2025

Тема диссертации доктора философии (PhD) по биологическим наукам зарегистрирована в Высшей аттестационной комиссии при Министерстве Высшего образования, науки и инновации Республики Узбекистан за номером B2024.3.PhD/B1238.

Диссертация выполнена в Центре передовых технологий при Министерстве высшего образования науки и инноваций Республики Узбекистан.

Автореферат диссертации размещен на трех языках (узбекском, английском и русском (резюме)) на сайте Научного совета cat-dscphd.uz и на информационно-образовательном портале «Ziyonet» (www.ziyonet.uz).

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Защита диссертации состоится «13» августа 2025 г. в 10^{00} часов на заседании Научного семинара по присуждению научных степеней DSC.03/30.07.2024.B.179.01 при Центре передовых технологий. Адрес: 100128, г.Ташкент, ул. Алмазарский район, Талабалар шахарчаси, 3A. Тел.: (+99871) 227 43 21

С диссертацией можно ознакомиться в Информационно-ресурсном центре Центра передовых технологий (зарегистрировано под № 4). Адрес: 100174, г.Ташкент, Алмазарский район, Талабалар шахарчаси, 3A. Тел.: (+99871) 227 43 21 (catscience@exat.uz).

Автореферат диссертации разослан: «_____» _____ 2025 г.

(реестр протокола рассылки № «1/7» от «11» июня 2025).

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ВВЕДЕНИЕ (реферат докторской диссертации)

Цель исследования. Основной целью исследования является разработка локальной рекомбинантной белковой вакцины против вируса SARS-CoV-2 в клетках CHO и определение её доклинической безопасности и иммунного профиля.

Объектами исследования были антигенсинтезирующие конструкции, экспрессированные антигены, клетки CHO, крысы линии Sprague Dawley и мыши линии Balb/c.

Научная новизна диссертационного исследования заключается в следующем:

Изучена экспрессия ряда антигенов SARS-CoV-2 в клетках CHO, при этом димерный белок RBD экспрессировался в больших количествах, чем корона-белки, прошедшие исследование. Определен выход димерного белка RBD после очистки, который составил 20–50 мг/л, с молекулярной массой около 50–55 кДа, в интактной и деградированной формах при экспрессии в клетках CHO. Методами жидкостной хроматографии достигнута очистка белка RBD в интактной форме до 99% и разработан протокол очистки.

Впервые разработана вакцина RENOVAC на основе рекомбинантного димерного белка RBD, полученного в клетках CHO с помощью генной инженерии, в двух концентрациях: 10 мкг/доза и 25 мкг/доза.

Вакцину RENOVAC вводили внутримышечно крысам линии Sprague Dawley в дозе 25 мкг, исходя из того, что наблюдаемые изменения в селезенке и тимусе были связаны с иммунологическим ответом на вакцину, не наблюдалось никаких неблагоприятных токсикологических изменений, а также что уровень не наблюдаемых неблагоприятных эффектов вакцины (NOAEL) составлял 25 мкг/дозу.

Введение вакцины внутрибрюшинно мышам Balb/c продемонстрировало хороший иммуногенный потенциал в низких и высоких дозах, но вакцина в дозе 10 мкг/дозу вызывала образование достаточного количества антител к RBD и вируснейтрализующих антител и поддерживала их высокий уровень в течение более длительного периода времени. Механизм действия вакцины

Впервые определен в Узбекистане, прежде всего она вызывает иммунный ответ путем активации CD4+ Т-хелперных клеток, и в свою очередь эта активация этих Т-клеток опосредует формирование гуморального иммунитета через активацию В-клеток, а изменение других показателей, таких как CD8+ клетки и интерлейкин-18, может активировать клеточный иммунитет после вакцинации.

Внедрение результатов исследований. На основе научных результатов, полученных в процессе создания и внедрения рекомбинантной белковой вакцины против вируса SARS-CoV-2:

В опытно-промышленном комплексе «CAT-Biotech» разработана малосерийная рекомбинантная белковая вакцина-кандидат против вируса SARS-CoV-2 (справка Министерства высшего образования, науки и инноваций Республики Узбекистан № 5/68 от 17 марта 2025 г.). В результате разработанная в ходе создания вакцины технология очистки белков методом жидкостной хроматографии позволила разработать;

Растворимый рекомбинантный белок S, полученный в клетках CHO, зарегистрирован Всемирной организацией здравоохранения (ВОЗ) на платформе разработки вакцин против COVID-19 (https://www.who.int/teams/blueprint/covid-19/covid-19-vaccine-tracker-and-landscape). В результате этого была разработана и проведена сравнительная оценка эффективности других вакцин;

Последовательность ДНК димерного белка RBD коронавируса SARS-CoV-2 зарегистрирована в базах данных GenBank и Национального центра биотехнологической информации (NCBI) под номерами PV232931 и XPR33336.1 (https://www.ncbi.nlm.nih.gov/nuccore/PV232931.1/). Это позволило улучшить и сравнить последовательности ДНК и белков при разработке новых вакцин.

Структура и объем диссертации. Диссертационная работа состоит из введения, 3 глав, заключения, списка литературы и приложений. Объем диссертации составляет 97 страниц.

E'LON QILINGAN ISHLAR RO'YXATI
СПИСОК ОПУБЛИКОВАННЫХ РАБОТ
LIST OF PUBLISHED WORKS
I bo'lim (I part, I часть)

1. Muzaffar Muminov, Nargiza Tsiferova, Vladimir Tsoy, Abrorjon Abdurakhimov and Shahlo Turdikulova. B cell epitopes of RENOVAC – dimer RBD protein subunit vaccine against COVID-19 // Uzbek Biological Journal – 2025 Feb. Volume 1, P.42-46 ISSN: 0042-1685 (03.00.00; №5).
2. Muminov M, Tsiferova N, Pshenichnov E, Ermatova K, Charishnikova O, Abdullaev A, Levitskaya Y, Dalimova D, Mvs S, Tomar G, Dewle A, Choudhari P, Wangikar A, Jadhav A, Mule M, Wangikar P, Abdurakhmonov I, Turdikulova S. Development, Pre-Clinical Safety, and Immune Profile of RENOVAC—A Dimer RBD-Based Anti-Coronavirus Subunit Vaccine. Vaccines. 2024 Dec;12(12):1420. https://doi.org/10.3390/vaccines12121420 P.1-21. ISSN: 2076-393X (№1 Web of Science/№3 Scopus Q1 Impact factor-5.2. H-index -85)
3. Turdikulova S, Dalimova D, Abdullaev A, Muminov M, Ruyue W, Pengfei L, Musabaev E, Yusupaliev B, Kurbanov B, Abdurakhmonov I. COVID-19 Response in Uzbekistan: From RT-PCR Test System to the Clinical Trial of Subunit Vaccine. In: Abdurakhmonov PIY, editor. COVID-19 Vaccines - Current State and Perspectives. Rijeka: IntechOpen; 2022. Available from: https://doi.org/10.5772/intechopen.109225 P33-44 ISBN: 978-1-80355-357-3 (№40 Research Gate).

II bo'lim (part II, II часть)

4. Н.А. Циферова, Д.А.Далимова, М.И. Муминов, Ш.Н. Ибрагимова, В.Э. Цой, С.С. Бозоров, О.С. Чарышникова, Ю.В. Левицкая, Ш.У. Турдикулова. Разработка рекомбинантной вакцины против коронавирусной инфекции на основе s-белка SARS-CoV-2 в республике Узбекистан // III Объединенный научный форум физиологов, биохимиков и молекулярных биологов научные труды том 2 Сочи – Дагомыс, Россия, 3–8 октября 2021 С.95
5. Muzaffar Muminov, Nargiza Tsiferova, Egor Pshenichnov, Husnora Ermatova, Oksana Charishnikova, Alisher Abdullaev, Yuliya Levitskaya, Dilbar Dalimova, and GLP lab team, Shahlo Turdikulova. Immune profile of dimer RBD-based anti-coronavirus recombinant protein vaccine// Book of abstracts of International Conference On "Biotechnology For Better Tomorrow" (BTBT-2024) organized by Microbiologists Society, India. Tashkent, Uzbekistan May 16th-17th 2024 P.5.
6. Muminov M., Tsiferova N., Pshenichnov E., Ermatova Kh., Charishnikova O, Abdullaev A., Levitskaya Y., Dalimova D., Sandhya M.,Geetanjali T., Ankush D., Pradhnya Ch., Aditi W., Jadhav A., Mrunal M., Pralhad W., Abdurakhmonov I., and Turdikulova Sh. Toxicity study of anti-COVID-19

dimer RBD vaccine - RENOVAC // International scientific conference of young scientists «Science and Innovation»: collection of scientific papers, Tashkent, Uzbekistan, November 15th 2024 P.46-47
7. Muminov M., Tsiferova N., Pshenichnov E., Ermatova Kh., Tsoy V., Charishnikova O., Abdullaev A., Levitskaya Yu., Dalimova D., Turdikulova Sh. Development of local dimer RBD anti-COVID-19 vaccine – RENOVAC // “Yangi, samarali biopreparatlar yaratishda bio va Nanotexnologiyalarning o‘rni” nomli xorijiy mutaxassislar ishtirokidagi ilmiy-amaliy anjuman materiallari Vaksina va Zardob instituti Toshkent sh. O’zbekiston. 27-noyabr 2024. S.104-105.

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