Problematika borreliových infekcí

- Modifikace povrchových antigenů Osp zajišťující zvýšení výtěžnosti exprese
- Návrh antigenů pokrývající svými epitopy široké spektrum variant jednotlivých genospecies
- Vývoj infekčního modelu na experimentálních myších
- Indukce imunitní odpovědi na slabé Osp antigeny aplikované formou liposomárních komplexů

Problematika borreliových infekcí

•Konstruován OspC bez lipidizačního signálu – zvýšení výtěžnosti

•Konstruována cDNA polyepitopu OspC, OspA, OspB nyní je testována imunogenita DNA vakcín a jsou připravovány proteinové vakcíny

•Vypracován infekční myší model p.e. navozené myší borreliózy

•Komplexy OspC vázané metalchelatační vazbou na liposomy komponentu s modulační komponentou non-MDP navozují vysoké titry specifických protilátek

Příprava rekombinantního nelipidovaného apyrogenního Outer surface proteinu C *Borrelia burgdorferi*

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2 Oddělení imunofarmakologie a imunotoxikologie, Výzkumný ústav veterinárního lékařství, Brno

Úvod

Outer surface protein C (OspC) je povrchový protein *Borrelie burgdorferi* – původce lymské boreliózy
Experimentální vakcinací rekombinantním OspC byla prokázána schopnost vyvolávat protektivní imunitní odpověď – slibný kandidát na konstrukci vakcíny proti lymské borelióze[1].
Na N – konci obsahuje lipidizační signální sekvenci o délce 18aa, tato sekvence je posttranslačně odštěpena a vzniklý N-terminální cystein je lipidizován připojením tří molekul mastných kyselin.
Naším cílem bylo zvýšit expresi rekombinantního proteinu úpravou jeho genové sekvence a ověření imunogenicity vzniklého produktu

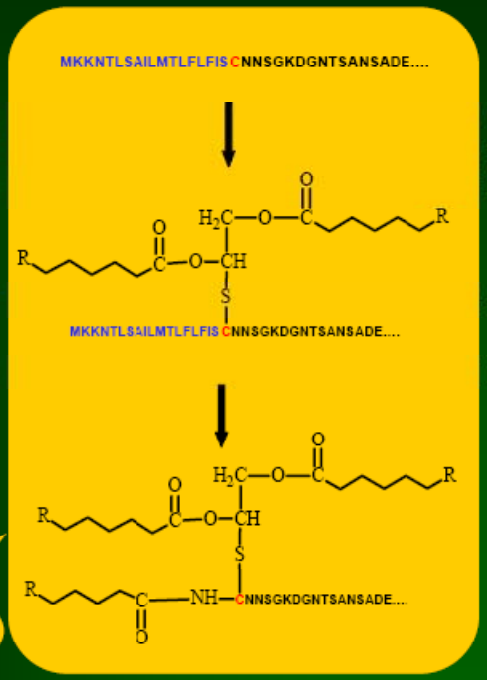


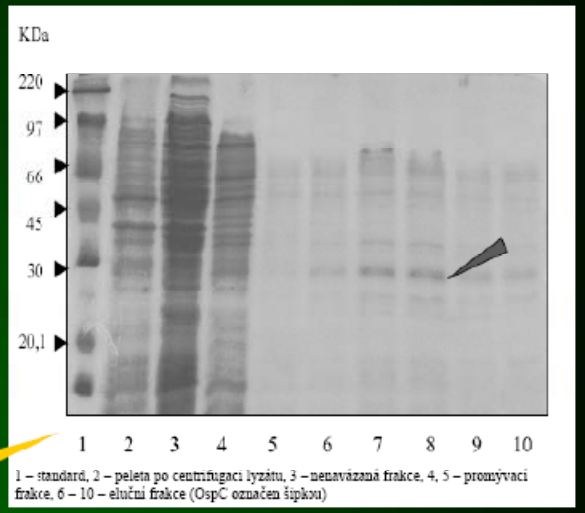
Schéma lipidizace OspC

Příprava nelipidované formy OspC

Pomocí PCR byla získána verze OspC zkrácená o prvních 54 nukleotidů. Rekombinantní OspC tak neobsahuje 18-ti aminokyselinovou lipidizační sekvenci. Byly použity primery upstream 5' – AGG TTT TIT TGG ACT TTC TGC C – 3' a downstream 5'- CAC CAT GTG TAA TAA TTC AGG GAA AGA TGG G – 3' Downstream primer obsahuje sekvenci pro vložení do plasmidu (bíle) a iniciační triplet (červeně).

Exprese a purifikace lipidovaného OspC

Gen pro OspC byl izolován z cDNA *Borrelia burgdorferi* a vnesen do vektoru pET28. K expresi byl použit bakteriální kmen Escherichia coli BL 21 (DE3) (Invitrogen). Kultivace probíhala v LB mediu při 37ºC, exprese byla indukována pomocí Isopropyl-β-D-galaktosidu (IPTG). Protein byl exprimován ve fúzi s His-tag a T7-tag pro následnou purifikaci a identifikaci. K purifikaci byla použita metaloafinitní chromatografie na NTA-agaróze s vázanými kovovými ionty (Ni^{2+}, Cu^{2+}, Co^{2+}).



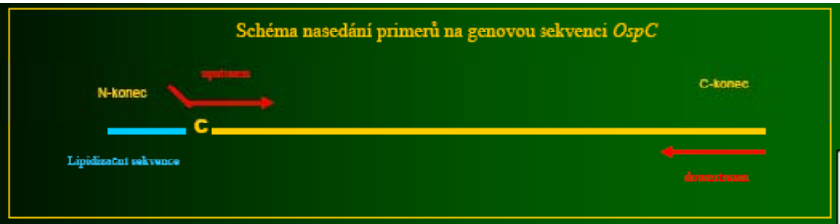
1 – standard, 2 – peleta po centrifugaci lyzátu, 3 – nenavázaná frakce, 4, 5 – promývací frakce, 6 – 10 – eluční frakce (OspC označen šipkou)

SDS – PAGE analýza purifikace OspC *B. burgdorferi*

Tímto postupem byl získán protein o nízké koncentraci a čistotě. K výrazněji lepšímu výsledku nevedly ani změny v postupu lýzy bakterií a purifikace. Lépe se osvědčila denaturační purifikace, která však může ovlivnit strukturu a tím i imunogenitu proteinu[2]. Využití dalších purifikačních kroků jako je iontově výměnná chromatografie či permeační chromatografie bránila nízká stabilita proteinu v používaných pufrech (PBS, Tris).

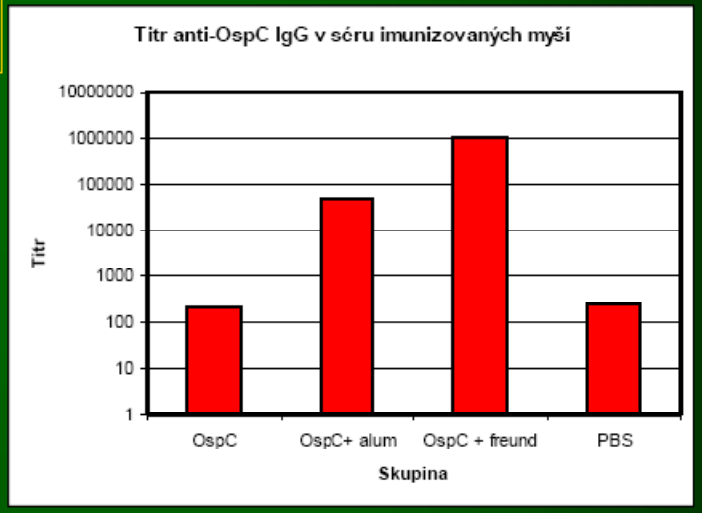
Depyrogenace OspC

Pyrogen (Lipopolysacharid) pocházející z buněčné stěny E. coli je častá kontaminace rekombinantních proteinů. V těle savců působí imunostimulačně až toxicky. V elučních frakcích bylo naměřena koncentrace více než 25.000 EU/ml (LAL test, Associates of Cape Cod Incorporated). Pomocí tří cyklů průtoku na depyrogenační koloně EndoTrap® Red (Lonza) byla hladina Lipopolysacharidu snížena na hodnotu pod 2,5 EU/mg proteinu.

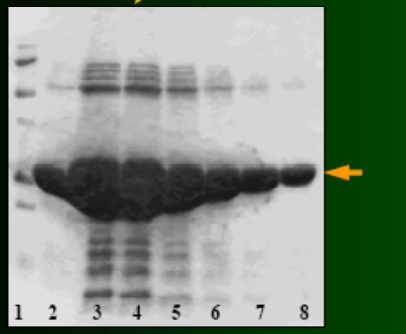


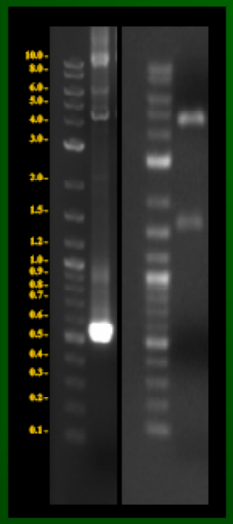
Imunogenita nelipidovaného OspC

DNA byla vyříznuta z agarózového gelu, purifikována pomocí Qiaex II kitu (Quiagen) a vložena do plasmidu pET101/D-TOPO (Invitrogen). Rekombinantní plasmid byl ověřen restrikční analýzou pomocí enzymu *EcoRV*. K expresi proteinu byly použity bakterie kmene E.coli BL21 (DE3). Protein byl exprimován ve fůzi s His-tagem a V5 tagem pro následnou purifikaci a detekci. Exprese indukovaná IPTG trvala 4 hodiny při 37ºC. Bakteriální biomasa byla lyzována nativní cestou. Lyzát byl použit k purifikaci pomocí afinitní chromatografie na Ni-NTA agaróze dle návodu výrobce (Qiagen). Sekvence proteinu byla ověřena pomocí MALDI-TOF (katedra biochemie přírodovědecké fakulty, data nezobrazena).

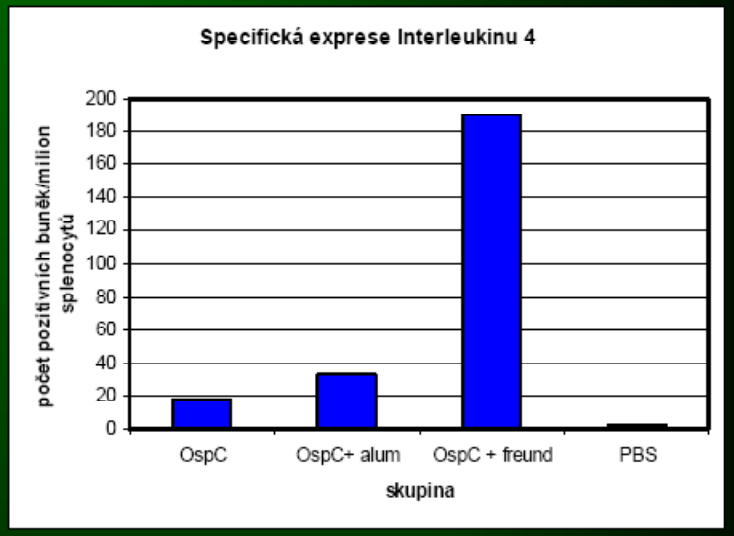


Skupinám myší Balb/c o šesti kusech a váze 18 – 20 g bylo podáno intradermálně 20 µg OspC v PBS, s Freundovým adjuvans, s hydroxidem hlinitým (alum) nebo samotné PBS. Vakcinace byla opakována po dvou týdnech. Byla sledována humorální odpověď metodou ELISA a buněčná odpověď pomocí detekce specifické produkce cytokinů ve splenocytech metodou ELISPOT. Metodou Western blot byla zjištěna pozitivní reakce s lyzátem *B. burgdorferi* (data nezobrazena).





Výsledek PCR Restrikce nukleasou *EcoRV*



Shrnutí a závěr

Lipidizovaný rekombinantní protein OspC *Borrelie burgdorferi* se v *E. coli* exprimuje s nízkou efektivitou a není stabilní

Odstraněním lipidizační sekvence z genu pro OspC došlo ke zvýšení výtěžnosti rekombinantního proteinu purifikovaného metaloafinitní chromatografií asi 50 x. Získaný protein je stabilní v běžně používaných pufrech.

Specifická exprese Interferonu gama

Problematika borreliových infekcí

LYMSKÁ BORELIÓZA – BIOLOGIE, PATOGENEZE, DIAGNOSTIKA A LÉČBA

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Ústav imunologie, Lékařská fakulta Univerzity Palackého, Olomouc

Práce shrnuje základní fakta týkající se patogeneze, diagnostiky, prevence a léčby lymské boreliózy. Toto multisystémové onemocnění je v současnosti nejčastější antropozoonózou na území Evropy a USA. Onemocnění postihuje nejen kůži, ale i nervový systém, pohybový aparát a kardiovaskulární systém. Původcem jsou spirochety z druhové skupiny *Borrelia burgdorferi*. Ačkoliv znalosti o nemoci za poslední roky zaznamenaly značné pokroky, některé aspekty zůstávají nejasné. Problém vakcinace také dosud nebyl vyřešen.
Klíčová slova: lymská borelióza, *Borrelia burgdorferi*, spirochety.

LYME DISEASE – BIOLOGY, PATHOGENESIS, DIAGNOSTICS AND TREATMENT
Paper reviews basic facts about pathogenesis, diagnostics, prevention and treatment of Lyme disease. This multisystemic disease is the most frequent zoonosis in Europe and the USA. The disease affects not only skin, but also nervous system, motoric apparatus, and cardiovascular system. Causative agent of the disease are spirochtetes from *Borrelia burgdorferi* species group. In spite of considerable research progress in recent decades, some features of this disease remain obscure. Similarly, the problem of vaccination is still unresolved.
Key words: Lyme disease, *Borrelia burgdorferi*, spirochaetes.

Dermatol. praxi 2008; 2(5–6): 236–239

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2007, 151(2):175–186.

BIOLOGICAL ASPECTS OF LYME DISEASE SPIROCHETES: UNIQUE BACTERIA OF THE *BORRELIA BURGDORFERI* SPECIES GROUP

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Received: September 15, 2007; Accepted: November 28, 2007

Key words: Borrelia burgdorferi/Lyme disease/Spirochete

Background: *Borrelia burgdorferi* sensu lato is a group of at least twelve closely related species some of which are responsible for Lyme disease, the most frequent zoonosis in Europe and the USA. Many of the biological features of *Borrelia* are unique in prokaryotes and very interesting not only from the medical viewpoint but also from the view of molecular biology.

Methods: Relevant recent articles were searched using PubMed and Google search tools.

Results and Conclusion: This is a review of the biological, genetic and physiological features of the spirochete species group, *Borrelia burgdorferi* sensu lato. In spite of a lot of recent articles focused on *B. burgdorferi* sensu lato, many features of *Borrelia* biology remain obscure. It is one of the main reasons for persisting problems with prevention, diagnosis and therapy of Lyme disease. The aim of the review is to summarize ongoing current knowledge into a lucid and comprehensible form.

Problematika mykotických infekcí

Uplatnění kandidového antigenu hsp90 u vaginální kandidózy

- Testování slizniční imunitní odpovědi na různé formy imunizace kandidovým hsp90 antigenem (zevní pohlavní cesty u experimentálních myší)
- Vztah efektorové a regulační imunitní odpovědi u VVC (přítomnost kandid versus přítomnost zánětu)
- Optimalizace myšího modelu VVC

Testování liposomárních dopravních systémů pro vakcinaci hsp90 proteinem

Problematika mykotických infekcí

Uplatnění kandidového antigenu hsp90 u vaginální kandidózy

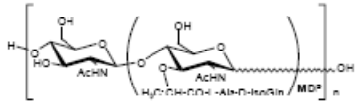
- Hsp90 antigen je rozlišován v průběhu VVC a imunizace zvyšuje intenzitu imunitní odpovědi
- Podle našich výsledků, na rozdíl od protektivity hsp90 antigenu u systémové infekce, u VVC nebyla protektivita prokázána (možná indukce tolerance?)
- Reorientace od hodnocení množství kandid k hodnocení subpopulací lymfocytů
- Nárůst imunitní odpovědi na vakcinaci rekombinantním hsp90/liposom plus MDP

NORMURAMYL GLYCOPEPTIDES AND LIPOSOMES FOR PREPARATION OF IMMUNOTHERAPEUTICS AND SYNTHETIC VACCINES

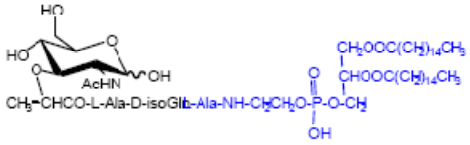
J. Turánek[1], J. Mašek[1], E. Bartheldyová[1], M. Ledvina[2], M. Raška[3] and A.D. Miller[4].

[1]Veterinary Research Institute, Brno, Czech Republic; [2]Institute of Organic Chemistry and Biochemistry Accademy of Science, Prague, Czech Republic, [3]Faculty of Medicine and Dentistry, University of Palacky, Olomouc, CZ; [4]Imperial College, Gene Therapy Centre, London UK.

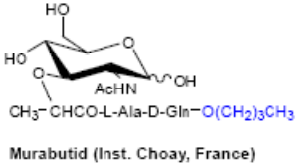
Analogs of MDP and GMDP developed as potential immunotherapeutics by pharmaceutical industry



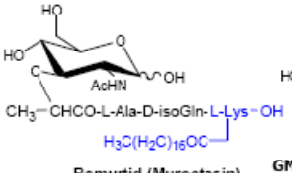
Peptidoglycan (n = 10 - 100)
MDP ("Muramyldipeptide", i.e. minimal immunoadjuvant unit)
GMDP (n = 1; "Glucosaminylmuramyldipeptide", i.e. basic repeating unit)



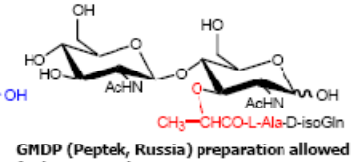
MTP-PE (Novartis-treatment of osteosarkoma)



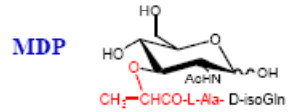
Murabutid (Inst. Choay, France)



Romurtid (Muroctasin)
(Daiichi Pharmaceutical Co., Japan)



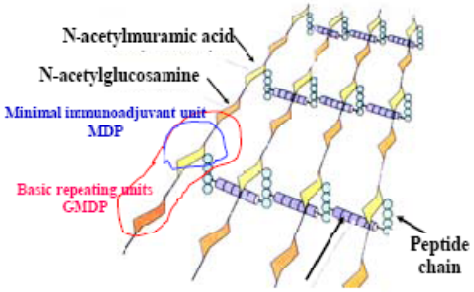
GMDP (Peptek, Russia) preparation allowed for human application



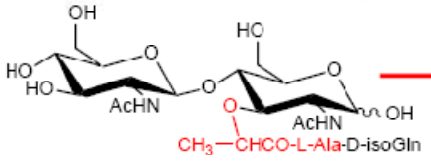
MDP

Transformations of MDP and GMDP molecules to norAbu-MDP and norAbu-GMDP

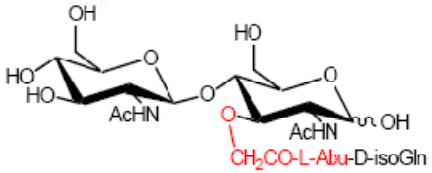
reduction or elimination the pyrogenicity and enhancement immunoadjuvant activity



Block scheme of the peptidoglycan of bacterial cell wall

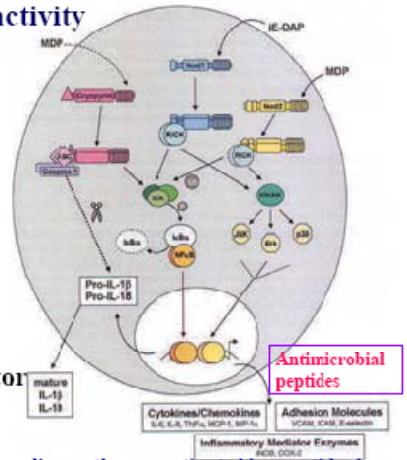


GMDP - Less pyrogenic and higher immunoadjuvant than MDP



norAbu-GMDP Nonpyrogenic and more immunoadjuvant than GMDP

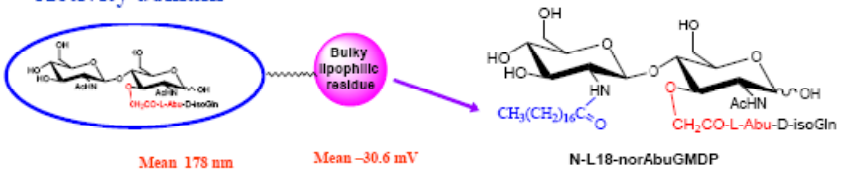
Hypothesis – low affinity of norAbu-MDP and norAbu-GMDP for Cryopyrin receptor prevents pyrogenicity and other side-effects induced by IL-1β



Signaling pathways activated by peptidoglycan-derived ligands

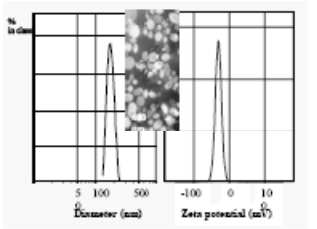
Liposomes - biocompatible carriers for immunoterapeutics and vaccines

Activity domain — **Accesory domain** –incorporation into lipid structures (**liposomes**, micelles, emulsions)



Mean 178 nm — Mean –30.6 mV

LiposIMM liposomal N-L18 norAbu-GMDP

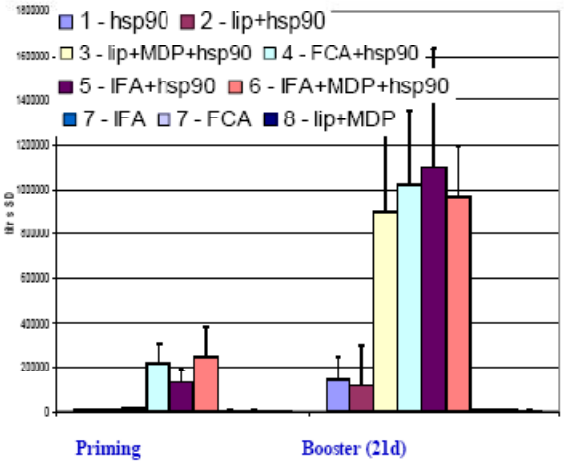


Applications

Synthetic vaccines

I.D. immunisation of mice with various formulations of rHSP90 antigen (MDP = lipophilic NorAbu-MDP analogue)

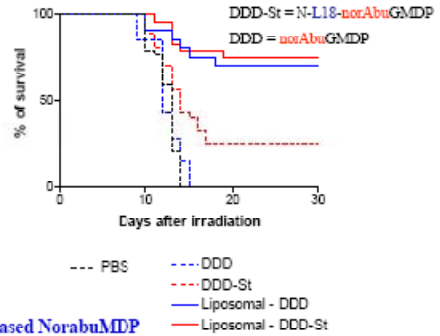
Antibody response



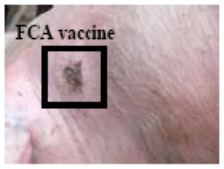
Stimulation of Innate Immunity

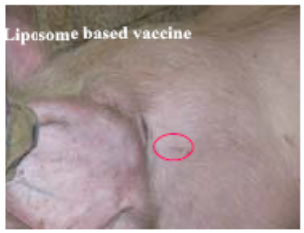
Survival curves of mice treated i.p. with free or liposomal immunomodulators 24 h before γ-irradiation (10 Gy).

J. Turánek, M. Ledvina et. al. *Int J Immunopharmac* 1997; 19: 611-7.



Safety of liposome-based NorabuMDP recombinant vaccines (PCV-2 vaccine) after i.d. application to pigs

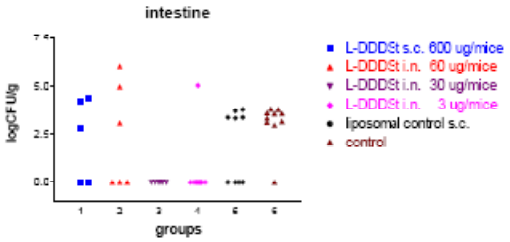




Stimulation of innate immunity of newborn kids against *Cryptosporidium parvum* by application of N-L18 norAbu-GMDP immunomodulator entrapped in liposomes

J. Turánek, M Ledvina, B Koudela, AD Miller et. al., *Parasitology* 2005, 131, 001-006

Stimulation of innate immunity against Salmonella infection in mice treated by i.n./p.o. route with liposomal N-L18-norAbu-GMDP



Problematika mykotických infekcí

REVIEW

Folia Microbiol. 52 (3), 291–312 (2007) http://www.biomed.cas.cz/mbu/folia/

Candidiasis – Do We Need to Fight or to Tolerate the *Candida* Fungus?

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ABSTRACT. Candidiases, infections caused by germination forms of the *Candida* fungus, represent a heterogeneous group of diseases from systemic infection, through mucocutaneous form, to vulvovaginal form. Although caused by one organism, each form is controlled by distinct host immune mechanisms. Phagocytosis by polymorphonuclears and macrophages is generally accepted as the host immune mechanism for *Candida* elimination. Phagocytes require proinflammatory cytokine stimulation which could be harmful and must be regulated during the course of infection by the activity of $CD8^+$ and $CD4^+$ T cells. In the vaginal tissue the phagocytes are inefficient and inflammation is generally an unwanted reaction because it could damage mucosal tissue and break the tolerance to common vagina antigens including the otherwise saprophyting *Candida* yeast. Recurrent form of vulvovaginal candidiasis is probably associated with breaking of such tolerance. Beside the phagocytosis, specific antibodies, complement, and mucosal epithelial cell comprise *Candida* eliminating immune mechanisms. They are regulated by $CD4^+$ and $CD8^+$ T cells which produce cytokines IL-12, IFN-γ, IL-10, TGF-β, *etc.* as the response to signals from dendritic cells specialized to sense actual *Candida* morphotypes. During the course of *Candida* infection proinflammatory signals (if initially necessary) are replaced successively by antiinflammatory signals. This balance is absolutely distinct during each candidiasis form and it is crucial to describe and understand the basic principles before designing new therapeutic and/or preventive approaches.

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Original Articles

Systemic and mucosal immunization with *Candida albicans* hsp90 elicits hsp90-specific humoral response in vaginal mucosa which is further enhanced during experimental vaginal candidiasis

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The *Candida albicans* heat shock protein 90 kDa (hsp90-CA) is an important target for protective antibodies in disseminated candidiasis of experimental mice and humans. Hsp90-CA is present in the cell wall of *Candida* pseudohyphae or hyphae – typical pathogenic morphotypes in both mucosal and systemic *Candida* infections. However, the potential protective effects of hsp90-CA-specific antibodies in vaginal candidiasis has not yet been reported. In the present study we used various vaccine formulations (recombinant hsp90-CA protein and hsp90-CA-encoding DNA vaccine) and routes of administration (intradermal, intranasal, and intravenous) to induce both hsp90-CA-specific systemic and vaginal mucosa immune responses in experimental BALB/c mice. The results showed that intradermal recombinant hsp90-CA protein priming, followed by intranasal or intradermal recombinant hsp90-CA protein boosting induced significant increases in both serum and vaginal hsp90-CA-specific IgG and IgA antibodies compared to the control group, as well as enhanced hsp90-CA-specific splenocyte responses *in vitro*. In the intradermally boosted group, subsequent experimental vaginal *Candida* infection induced additional increases in the hsp90-CA specific IgG isotype, suggesting that *Candida* has the ability to induce a local hsp90-specific antibody (IgG) response during vulvovaginal candidiasis. Further work is required to elucidate the importance of immunity to highly conserved antigens during infection of the human female reproductive tract where a balance between immunity to and tolerance for commonly antigens such as hsp90 is necessary for the maintenance of fertility.

Keywords candidiasis, hsp90, antibody, recombinant protein vaccine, DNA vaccine

Problematika mykotických infekcí

Příprava vakcinačních antigenů Trichophyton spp.

- Využití DNA vakcíny hsp60 ke studiu eliminace DNA vakcíny z organizmu imunizovaného jedince
- Izolace hsp60 a CDO cDNA v plné délce pro přípravu rekombinantních antigenů

Problematika mykotických infekcí

Genetic Vaccines and Therapy

BioMed Central

Research

Open Access

Quantitative real-time PCR study on persistence of pDNA vaccine pVax-Hsp60 TM814 in beef muscles

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Abstract

Background: Application of plasmid DNA for immunization of food-producing animals established new standards of food safety. The addition of foreign products e.g. pDNA into the food chain should be carefully examined to ensure that neither livestock animals nor consumers develop unpredicted or undesirable side-effects.

Methods: A quantitative real-time PCR (QRTPCR) methodology was developed to study the biodistribution and persistence of plasmid DNA vaccine pDNAX (pVAX-Hsp60 TM814) in mice and beef cattle. The linear quantification range and the sensitivity of the method was found to be 10 – 10^9 copies per reaction (500 ng/gDNA) and 3 copies per reaction, respectively.

Results: Persistence of pDNAX in mice muscle tissue was restricted to injection site and the amount of pDNAX showed delivery formulation dependent (naked pDNA, electroporation, cationic liposome complexes) and mouse age-dependent clearance form injection site but pDNAX was still detectable even after 365 days. The QRTPCR analysis of various muscle tissue samples of vaccinated beef bulls performed 242–292 days after the last revaccination proved that residual pDNAX was found only in the injection site. The highest plasmid levels (up to 290 copies per reaction) were detected in the pDNAX:CDAN/DOPE group similarly to mice model. No pDNA was detected in the samples from distant muscles and draining lymph nodes.

Conclusion: Quantitative real-time PCR (QRTPCR) assay was developed to assess the residual pDNA vaccine pVAX-Hsp60 TM814 in mice and beef cattle. In beef cattle, ultra low residual level of pDNA vaccine was only found at the injection site. According to rough estimation, consumption of muscles from the injection site represents almost an undetectable intake of pDNA (400 fg/g muscle tissue) for consumers. Residual plasmid in native state will hardly be found at measurable level following further meat processing. This study brings supportive data for animal and food safety and hence for further approval of pDNA vaccine field trials.

REVIEW

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DNA vaccines: are they still just a powerful tool for the future?

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Abstract

Vaccination is historically one of the most successful strategies for the prevention of infectious diseases. For safety reasons, modern vaccinology tends toward the usage of inactivated or attenuated microorganisms and uses predominantly subunit vaccines. The antigens need to be clearly defined, pure, stable, appropriately composed, and properly presented to the immune system of the host. Differing ratios of various proportions between specific $CD4^+$ and $CD8^+$ T cell responses are essential for conferring the required protection in the case of individual vaccines. To stimulate both $CD4^+$ and $CD8^+$ T cells, the antigens must be processed and presented to both antigen-presentation pathways, MHC I and MHC II. Protein antigens delivered by vaccination are processed as extracellular antigens. However, extracellularly delivered antigen can be directed towards intracellular presentation pathways in conjugation with molecules involved in antigen cross-presentation, e.g. heat shock proteins, or by genomic-DNA vaccination. In this overview, current knowledge of the host immune response to DNA vaccines is summarized in the introduction. The subsequent sections discuss techniques for enhancing DNA vaccine efficacy, such as DNA delivery to specific tissues, delivery of DNA to the cell cytoplasm or nucleus, and enhancement of the immune response using molecular adjuvants. Finally, the prospects of DNA vaccination and ongoing clinical trials with various DNA vaccines are discussed.

Key words: DNA vaccine, plasmid, delivery systems, CTL, MHC I, MHC II.

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Problematika HIV infekce

Studium alternativních vakcinačních přístupů a studium glykosylace Env antigenu

- Současná DNA vakcinace hlavně i.m. málo efektivní u člověka
- Studium efektu DNA vakcinace do tkání s vysokou mírou proteosyntézy
- Modelové srovnání glykosylace Env antigenu v závislosti místu aplikace DNA
- Využití imunomodulačních molekul k zvýšení imunitní odpovědi na HIV antigeny
- Příprava rekombinantních proteinových vakcín p24 konjugovaných s hsp proteiny (hsp70, grp78, hsp90, gp96)

Problematika HIV infekce

•Indukce intenzivní a dlouhodobé antigenně specifické imunitní odpovědi na aplikaci Env DNA vakcíny do hepatocytů

•Postup využitelný pro indukci specifické intenzivní mukózní odpovědi

•Glykosylace Env antigenu je závislá na buněčné linii použité pro jeho expresi

•Tkáňový původ Env exprimujících linií významně ovlivňuje vazbu neutralizačních protilátek

•DNA vakcinace (p24) gag antigenem společně s imunomodulačními molekulami OD40-L nebo 41BB-L zvyšuje významně intenzitu imunitní odpovědi

•Přípraveny rekombinantní proteiny p24 s hsp70 v několika formách

Influence of immunization sites and the glycosylation of HIV-1 envelope glycoprotein gp120 on the effectiveness of a DNA-based vaccine

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Abstract

The high incidence of HIV-1 infections requires novel approaches for prophylactic vaccination to induce protective responses. Based on our data with a DNA vaccine encoding gp120 of HIV-1, liver targeting induced higher titers of specific antibodies (Abs) than intramuscular injection. To determine whether these differences were due to the high rate of proteosynthesis in the liver, or rather due to differential glycosylation of gp120 by hepatocytes and muscle cells, we transfected a human hepatoma cell line (HepG2) and a human rhabdomyosarcoma cell line (RD) with HIV-1 Env DNA and characterized in vitro and in vivo the gp120 produced by these cell lines. As controls, we used human embryonic kidney cells (293T) and Chinese hamster ovary (CHO) cells. Using gas-liquid chromatographic and western blot (WB) analyses, we found significant differences in the carbohydrate composition (content of mannose, N-acetylglucosamine, galactose, and fucose moieties) of gp120 produced by different cell lines. When mice were intradermally immunized with purified Env glycoproteins from these cell lines, the levels of Env-specific Abs in mouse sera, determined by ELISA and WB, were comparable. However, the gp120 isolated from HepG2 cells induced serum Abs with higher HIV-neutralizing capacity. Our data show that changes in glycosylation can affect the resulting Abs responses and the vaccine efficacy. Therefore, targeting of HIV-1 DNA vaccines to the liver through various delivery systems can result in quantitatively and qualitatively superior immune responses.

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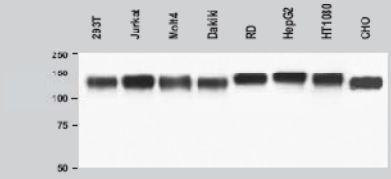
Figure 1
Molecular weight of HIV-1 ConB gp120+MBL protein expressed in various cell lines



Above indicated cell lines were transfected with plasmid encoding for HIV-1 ConB gp120+MBL under CMV control. Expressed protein was separated on SDS-PAGE, blotted and stained with antibody against V5 tag which was fused to the C'end.

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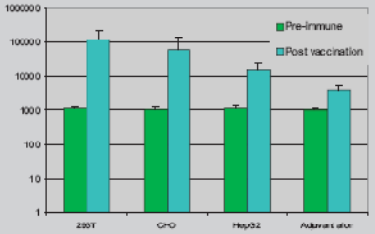
Figure 2
Monosaccharide compositional analysis of recombinant HIV-1 ConB gp120+MBL produced in different cell lines

Sugar	HepG2	RD	293T	CHO
Fuc	0.33	0.08	0.21	0.17
Man	2.16	1.39	3.21	2.32
Gal	1.02	0.42	0.78	0.63
Glc	0.05	0.13	0.19	0.10
Internal Std	1.00	1.00	1.00	1.00
GlcNAc	1.74	0.59	2.08	1.27
GalNAc	0.13	ND	0.12	0.08
SA	0.89	0.31	0.59	0.66

Data are expressed as response factors (average from two experiments relative to standard sugars and normalized to internal standard).
ND, not detected; Fuc, fucose; Man, mannose; Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; SA, sialic

Figure 3
HIV-1 gp120-specific antibodies in sera of i.d. immunized mice



Mice (five per group) were injected i.d. twice with 10 μg of recombinant HIV-1 ConB gp120+MBL protein expressed in different cell lines (293T, CHO, HepG2). (MBL is responsible for multimerization of gp120 Raska et al, Vaccine 2008). Result generated by ELISA using ConB gp120 produced in 293T cells as capture, are expressed as mean end-point +/- SD. Green bars represent pre-immune sera and blue represent sera after second immunization.

HIV-1 ConB gp120 antigen used:

Cell lines used in study:

293T	human embryonic kidney cell line
CHO	chinese hamster ovary cell line
HepG2	human hepatocellular carcinoma cell line
Jurkat	human T cell line (T cell leukemia)
Molt4	human T cell line (ALL)
Dakiki	human B cell line (EBV transformed)
RD	human rhabdomyosarcoma cell line (muscle)
HT1080	human fibrosarcoma cell line

Figure 4
HIV-1 virus-specific antibodies in sera of immunized mice

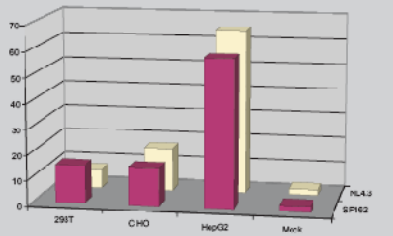
A

B

Mouse Group	End-point titer ELISA: LabSystems HIV EIA	Calypte HIV urine kit	V3 loop (Mork)
293T	50 (15)	64,000	16,000
CHO	66 (34)	32,000	16,000
HepG2	68 (24)	64,000	16,000
Naive	< 10	8,000	2,000

(A) WB strips (Cambridge Biotech) developed with pooled sera of mice immunized as indicated in Fig. 3.(B) End-point titers measured in sera of immunized mice using either LabSystems HIV EIA [mean x 1000 (SD)], or Calypte HIV urine kits or a preparation of V3 loop.

Figure 5
HIV-1 neutralization by sera of immunized mice



Sera from mice immunized as in Fig. 3 were tested for neutralization of HIV-1 (NLA4.3 or SF162) viruses using JC53-BL indicator cells. To stress different neutralization activity the neutralization titers are divided by end-point titer determined in each group.

Conclusions

1) Differential glycosylation was observed in HIV-1 gp120 antigen expressed in different cell lines

2) Neutralization activity of mouse sera was affected by the cell type used for gp120 expression

3) Glycosylation pattern of gp120 antigen generated by various tissues needs to by analyzed to assess the relevance of particular DNA vaccination route.

Results

Equal amounts of recombinant gp120+MBL obtained from four cell-types were separated by SDS-PAGE, electroblotted onto PVDF membranes, and then tested with a panel of V3-directed mAbs or non-V3 control Abs (Chessie 13 or anti-V5) for epitope recognition. In this pilot study, selected mAbs revealed significant differences in their capacity to recognize the V3 loop of gp120 dependent on the source cell-type (**Fig. 3**). Specifically, gp120+MBL produced in HepG2 cells was recognized by most of the mAbs studied, while Gp120+MBL produced in 293T, Jurkat, and CHO cells was poorly recognized by two, two, and three mAbs of the six-member panel, respectively.

Using gp120+MBL produced by the same four cell-types, but using also mAb 39F and polyclonal goat anti-gp120 antibody, a follow-up study was performed with altered experimental conditions (**Fig. 4**). Membranes were first probed with anti-V5 to normalize gp120+MBL (load control). The membranes were then stripped, checked for completeness of stripping, and then re-probed with the mAb panel. The western blots shown in **Fig. 4** were densitometrically analyzed and data were expressed as a ratio of their V3 mAb ECL response relative to the response established with anti-V5 (**Fig. 5**).

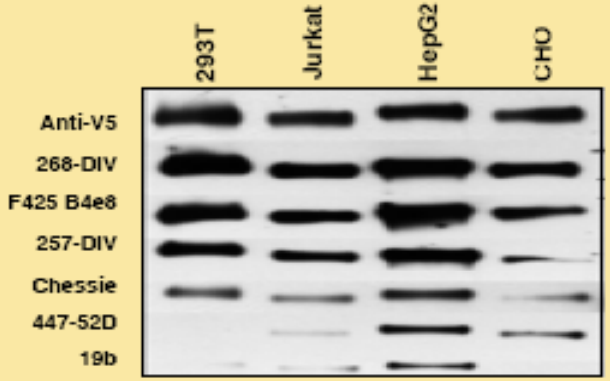


Figure 3. Pilot study of gp120 recognition by V3-specific mAbs: a comparison of mAb (side) recognition of recombinant gp120+MBL produced by different cell-types (top). Ordered from least to greatest difference with respect to anti-V5 mAb normalization.

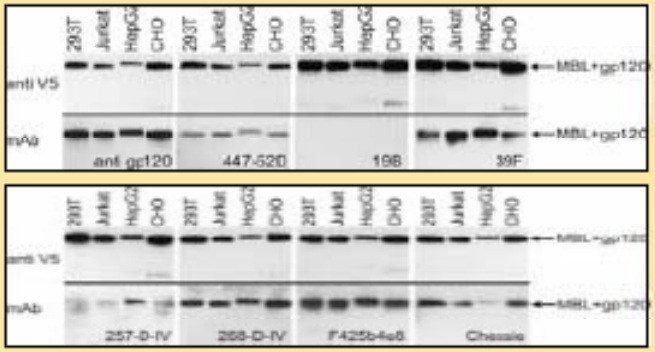


Figure 4. Follow-up study of mAb panel with the addition of 39F and polyclonal gt anti-gp120. Above: the amount of MBL+gp120 visualized with anti-V5 mAb used to normalize protein load. Below: binding of V3-specific mAb after stripping.

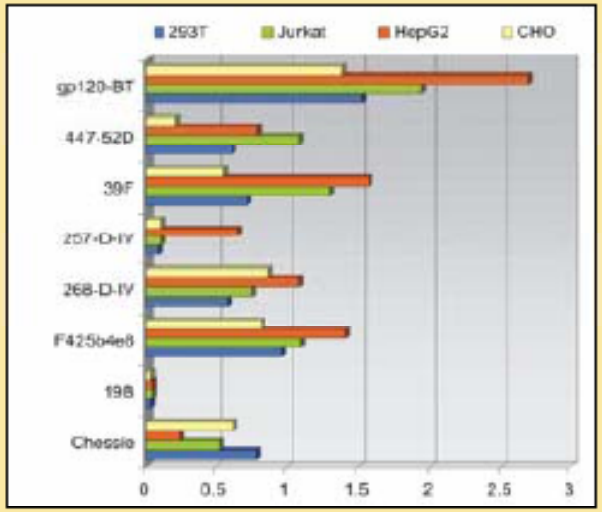


Figure 5. Plot of ECL-visualized bands after addition of mAbs in rapport to the bands developed with anti-V5. Of most interest is the different recognition of gp120+MBL produced in different cell-types when tested with the same V3 mAb.

To assess applicability of this research to live-virus models, a competition assay was performed using live, recombinant HIV-1 viruses and the JC53-BL indicator cell-line containing reporter cassettes for luciferase and β-galactosidase, each independently expressed from an HIV-1 long terminal repeat. Serially diluted gp120 preparations from 293 T cells were mixed with the HIV-1 pseudoviruses SF162 and NL43. The reduction in luciferase production indicated the reduced access of the recombinant virions to the receptor/co-receptor on the cell surface. The results are expressed as the percent reduction of viral infectivity in sample as compared to the control (virus alone).

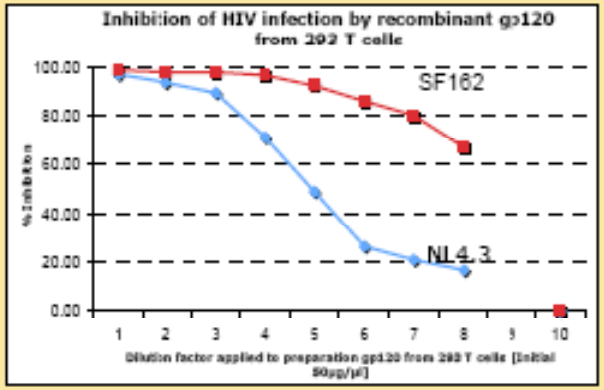


Figure 6. Inhibition of HIV-1 infection by recombinant gp120+MBL expressed in 293T cells. Gp120+MBL added together with the HIV-1 pseudoviruses SF162 and NL4.3 inhibits HIV-1 infection in the JC53-BL indicator cell-line in a dose-dependent manner.

Problematika HIV infekce

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ScienceDirect

journal homepage: www.elsevier.com/locate/vaccine

Delivery of DNA HIV-1 vaccine to the liver induces high and long-lasting humoral immune responses

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KEYWORDS
DNA vaccine;
Liver targeting;
HIV-1 Env immune responses

Summary The quality of immune responses induced by DNA vaccination depends on the site of DNA administration, the expression, and the properties of the encoded antigen. In the present study, we demonstrate that intravenous hydrodynamic HIV-1 envelope DNA injection resulted in high levels of expression of HIV-1 envelope antigen in the liver. When compared to the administration of DNA by i.n., i.d., i.m., and i.splenic routes, hycrodynamic vaccination induced, upon DNA boosting, levels of HIV-1 envelope-specific antibodies 40-fold higher than those elicited by the other routes tested. Hydrodynamic vaccination with 1 μg DNA induced higher humoral responses than 100 μg DNA given intramuscularly in the prime-boost regimen. High levels of envelope-specific IgG and IgA antibodies were induced in genital tract secretions after two doses of DNA followed by intranasal boosting with recombinant HIV-1 gp120 protein. Furthermore, two doses of 100 μg DNA generated interferon-gamma production in ~4.3 ± 1.7% of $CD8^+$ splenocytes after *in vitro* stimulation with HIV-1 envelope peptides. These results demonstrate that DNA vaccines targeted to tissues with high proteosynthetic activity, such as the liver, results in enhanced immune responses.

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Novel Modification of Growth Medium Enables Efficient *E. coli* Expression and Simple Purification of an Endotoxin-Free Recombinant Murine hsp70 Protein

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Heat shock protein 70 kDa (hsp70), a molecular chaperone involved in folding of nascent proteins, has been studied for its ability to activate innate and specific immunity. High purity hsp70 preparation is generally required for immunization experiments, because endotoxins and other immunologically active contaminants may affect immune responses independently of hsp70. We have developed a novel modification of *E. coli*-expression medium that enabled a simple two-step production and purification method for endotoxin-free recombinant hsp70. During Ni–NTA-based affinity purification of hsp70, a contaminating protein from host *E. coli* cells, L-glutamine-D-fructose-6-phosphate aminotransferase (GFAT), was identified. By testing various compounds, supplementation of growth medium with a GFAT metabolite, *N*-acetylglucosamine, was found to reduce GFAT expression and increase the total hsp70 yield five times. The new protocol is based on column purification of His-tagged hsp70 protein produced by *E. coli* with the modified medium, followed by endotoxin removal by Triton X-114 extraction. This approach yielded hsp70 with high purity and minimal endotoxin contamination, making the final product acceptable for immunization experiments. In summary, a simple modification of growth medium allowed production of recombinant mouse hsp70 in high yield and purity, thus compatible with immunological studies. This protocol may be useful for production of other His-tagged proteins expressed in *E. coli*.

Heat shock proteins (hsp) have been described to stimulate T cell-mediated immunity as well as the specific antibody responses against the coupled polypeptides [19, 20]. In this process, the hsp moiety is responsible for the receptor-mediated binding of hsp–peptide complex, and delivery of peptide to the proteasome processing followed by presentation of peptide fragments on MHCI molecule to host cytotoxic $CD8^+$ T cells [8, 18].

Isolation of recombinant hsp is the starting point for their testing as potential modulatory molecules in anticancer or anti-infectious disease treatment [6, 7, 23]. However, the production and purification of hsp is a principal limitation for those applications. Tagging of recombinant proteins with a His epitope facilitates affinity purification of the expressed protein using Ni(II), Co(II), or Cu(II) ions on Ni–NTA resins. However, Ni–NTA affinity columns additionally bind other histidine-rich proteins. Removal of such impurities can be achieved by a combination of affinity chromatography with separation techniques, consequently increasing the cost of the product.

Here, we report a simple medium manipulation that enabled isolation of a full-length recombinant mouse hsp70 protein expressed in *E. coli* using a simple two-step purification approach. We noticed that on the Ni–NTA column, the hsp70 was co-purified with L-glutamine-D-fructose-6-phosphate aminotransferase (GFAT; E.C. 2.6.1.16), which has physicochemical properties similar to those of