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PROTEIN FACTORS REGULATING THE HUMAN DEFENSE CELLS: INVOLVEMENT OF GLYCOCONJUGATES

Lakhtin V. M.*, Lakhtin M. V. and Aleshkin V. A.

G.N. Gabrichevsky Reseach Institute for Epidemiology and Microbiology, Moscow 125212, Russia.

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***Corresponding Author**
Lakhtin V. M.
G.N. Gabrichevsky Reseach Institute for Epidemiology and Microbiology, Moscow 125212, Russia.

ABSTRACT

An analysis of the influence of glycoconjugates and carbohydrate moiety on protein factors modulating the activity of protective cells was carried out, including emphasis on the current state of problems in the relationship of protein macrophage-activating factors (MAFs). The prospects of using synthetic polymer glycoconjugates using the patterns of protein/glycoprotein-glycoconjugate interactions established by the authors and based on highly sensitive microanalysis methods developed with the participation of glycoconjugates of mono- and assembly molecular targets associated with membranes in the study of prophylactically and therapeutically significant (glyco)(lipo) proteins and (glyco)(lipo)peptides, including those affecting cellular population communications on the examples of MAFs and their peptide derivatives (beta-lactoglobulin, GcMAF, immunoglobulins, others) of colostrum, milk and probiotic cultures, are considered.

KEYWORDS: Cell activation factors, MAF, GcMAF, beta-lactoglobulin, macrophages, glycoconjugates, glycoproteins, recognition, assembly, protection, multiple forms, lectinbiotics, enzymebiotics.

ABBREVIATIONS

GC glycoconjugates

GcMAF Gc protein ((human group-specific component, a vitamin D-binding protein) derived MAF

GP glycoproteins

MAF macrophage-activating factor(s)

MIF migration inhibitor factors

Mp macrophages

PAA polyacrylamide

1. INTRODUCTION

It is relevant to study the network biological activities of populations of protective cells and their subpopulations from the point of view of the positive effect of the protein factors of colostrum[12,17,24-26,30,42,71, 60, 63, 65,69,78, 81], milk[22,25,73,80,82], culture fluids and the cell surface of probiotics and other fermented milk bacteria-commensals[4-7,9,35,48,54,55,58,62,83,87] on them with the result of strengthening the body's immunity.[1,2,11,37,44,60] At the same time, there is an obvious need for systemic multifactorial approaches in the study of complex (combined and cascaded) biological activities of protective cells, including (sub)populations of macrophages (Mp) of the peritoneum and Mp-like blood cells[1,3,7,8,10,11,29,37,41,52,55,58,62,81,83] Glycoconjugates (GC) play a special role in molecular and cellular communications of protection)[3-9,50,52,53] Their consideration can reveal a new potential of beneficial properties for humans at the supracellular, surface cell and intracellular (cytoplasmic and organelle) levels of the unified protection of the body from infections. However, this aspect remains the least studied.

The *goal* is to conduct a analysis of the potential of GC in the study, modulation and navigation of protein factors influencing the cells of the body's defense, including Mp, systemic GC as an important constituents of the overall adaptable defense of the body, based on our data.

2. THE MAIN CONTENT

2.1. *Aspects of the GC using in glycobiology*

GC include such carbohydrate-containing natural compounds as (lipo)polysaccharides, glycolipids, glyco(lipo)proteins (GP); their degradation products; as well as synthetic analogues of these natural categories. In this case, GC with a covalently attached carbohydrate part in the molecules are considered as containing a carbohydrate domain with additional functions such as modulating the communication activity of cells, the implementation of synergistic or antagonistic/ inhibitory combined reactions and resulting attacks, the manifestation of direct or indirect (mutual)actions against pathogens, pathogenic microbiocenoses, in support of non-antibody and antibody protection systems, innate and acquired immunity. GC can be submitted (and considered) systems of multiple forms, including combined ones, with varying biological activities, the manifestation of which becomes even more diverse (the stability of the protective response, the response to stress is achieved) as part of complexes with other effectors and (co)factors - protein and non-protein, peptide and (glyco)(lipo)peptide nature. Such systems are characterized as co-functioning with a labile (navigationally configurable) system vector of potential resultant action/ activities (combined activities).

The keys (according to our data) to the use of polymer synthetic GC as tools for the study of proteins, GP and receptors.[3-7, 9, 50-53]

Linear polymer based on the polyacrylamide (PAA) soluble in water and aqueous salt solutions were used-bark with lateral branches of carbohydrates (25% by weight) Gc (www.lectinity.com), imitating "mucins"-like polyvalent compounds with multiple clusters/sites of exposed homogeneous carbohydrate residues in the form of short (1-3 residues) lateral antennas. Such GC adapt their conformation ("random clumping" in solution) in accordance with the characteristics of carbohydrate-accepting protein sites, GP (and other types of GC) and their assembly complexes on the solid phase (membranes) simulating the cell surface. The GC used in the work imitated natural compounds of fundamentally different origin and could be used as natural models of reactions and processes in the body.

We have obtained the following results and conclusions about the patterns of interactions (glyco)(lipo)protein and synthetic polymer GC systems - soluble and solid-phase/surface-cell (in aqueous and solid phases):

*synergism of the action of GC among themselves and with antibodies;

*the ability of selected several (2-3) types of GC to visualize some/certain forms of protein factor as much as possible, to map the features of its structure;

*the ability of synergistic sets of GC to recognize and bind protein factors /cytokines in the compositions of molecular complexes, nano- and microparticles;

*preservation of factors in the composition of functionally active complexes and nanoparticles of the ability/activity for further sequential cascade binding of different types of GC;

*sensitivity of GP targets to the topography of antenna clusters exposed in GC (the ability of the protein to recognize the availability and distribution in the space of clusters and antennas of glycans);

*the presence of GC in the spatial field as systems (there is at least one “virtually binding” parameter of the space coordination);

*asymmetry (generalized directionality and vectorality), mosaic systems of multiple forms of GP, including as complexes (their disparity in the severity of modulation of activities);

*expanding the potential of GC recognition by therapeutic protein/GP, the ability to form new recognition sites in protein-based complexes and supramolecular ensembles;

*correspondence between the systems "GC Recognition Proteins" and GC as a "One-Partner system/subsystem" (One GC is a set of proteins, One protein is a Set of GC), the presence of correspondence between protein and GC subsystems, the establishment of ranking orders (sub)Gc and protein systems, depending on the implementation of the types of activities;

*identification of the probiotic potential of the studied GC to support probiotic microbiocenoses, including those that increase the biocompatibility of lactobacilli, bifidobacteria, and their mixtures.

2.2. ***Basic properties of the protein factors influencing human protective cells*****[10,39,40,56,68,74]**

Protein MAFs exhibit all the properties of cytokine families, are, as a rule, GP and represent a set / system of multiple forms of various biological/ physiological purposes. Examples of some of the most studied protein factors (also as derivative effector peptides) are beta-lactoglobulin.[16,20,21,27,28,32,36,45,46,66,70,77,86], GcMAF[10,39,40,56,68,74], other types of milk proteins.[72,73] As a rule, the properties and main forms of MAF are related (largely determined) by purification methods, for example, GcMAF using immobilized vitamin-D, since valuable antiviral properties are revealed in the line of anti(vitamin D/D3)-binding proteins.[15,31,34,56,75,76,86] The microheterogenicity of the forms is determined not only by the action of environmental enzymes (including the effect of limited proteolysis), but is largely due to the presence of a highly variable carbohydrate part of the cytokine.[24,25] The predominant forms corresponding to the specific responses of the Mp subpopulations are distinguished.[10,11,15,24,25]

2.3. ***Biological activities of the MAFs***

The main, most pronounced and studied protein factors affecting the activity of Mp include variants of MAFs, migration inhibitor factors (MIFs), chemotaxis reagents and other proteins, GP and their peptide derivatives - all of preventive and therapeutic significance, expanding the network of co-functioning between populations of Mp, Mp and other protective cells, tissues and organs, thereby increasing the reliability, stability and reactivity of the responses of the unified defense of the body. MAF activities are modulated by hydrolases present in the environment (including participants in the probiotic compartment of biotope microbiocenoses), which strengthen or weaken the vector of the initial action of the protein factor at the peptide level.[5-7,16,23,34,36,47,49,57,64,66,67,72,79,84]

2.4. *Prospects for the discovery of new properties of MAFs, including taking into account interactions with GC, include* (the basic aspects/ sides/ components of the issue are cited)

-genetic engineering aspects of studying and obtaining glycosylated MAFs[24, 25];

- Lectin properties of MAFs, MAFs as lectinbiotics[7,9,21,50,51,53,77], lectin sites in GC recognition during assembling milk protein factors[20,21,45,77];

- The relationship of MAFs with enzymes and enzymbiotics[53,73];

- MAF-regulation of biomarker enzymes in the serum of patients (increased activity of N-acetylgalactosaminidase - nagalase, which completely - not selectively deglycosylates vitamin D-binding protein and, thereby, blocks the formation of GcMAF, which leads to immune disorders)[15,19];

- (glyco)(lipo)targeted peptides (including products of limited proteolysis, including those involving hydrolase systems of probiotics and/or strains and mixtures of lactic acid bacteria-comensals;

-postbiotic aspects (MAFs, including GcMAF, as sources and regulators of systemic postbiotic products, in synergistic combinations)[5,6,83,87];

- directed purification of the "systemic MAFs" (through selected combined stages of affinity chromatography, with the achievement of a combined endogenous biocompatible/stable composition with the desired resultant action/actions);

- combined compositions of MAFs with additives, including probiotic and/or hydrolase nature;

- modulation of MAF communications through the formation of complexes with GP:

-- (inter)molecular, (inter)cellular, network;

- preventive and therapeutic aspects in relation to COVID-19 and concomitant diseases[6-8,15,38,48,61,74-76,82], infectious diseases of microbial nature[13,18,71,78,80]; in connection with tumors[10,15,19], obesity and other metabolic disorders[6,7,40,54,78];

--the use of GC/ oligosaccharides/ polysaccharides of milk and probiotics (their cultural hydrolysates) for disease-preventing purposes.[1,13,18,19,29,33,43,59]

In connection with the fight against COVID-19, research and purification of angeotensin-I-converting enzyme inhibitors are becoming important.[14,15,35,85]

3. CONCLUSION

These data indicate a significant potential of GC in modulation, navigation and Mp training. The use of GC, the regularities of protein-GC interactions and GC-based technologies in the study of functionally active therapeutic proteins and factors on the examples of MAFs in aspects of immunology, biotechnology (microbiology), pharmacology, prevention and therapy of diseases is promising.

Disclosure of conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Blyakher MS, Aleshkin AV, Fedorova IM, Ramazanova ZK, Krasnopolskaya LM, Tulskaya EA, Kukushkina EA. The ability of human peripheral blood mononuclears to produce cytokines when exposed to xylomannom *in vitro*. *Medical immunology,* 2017; 19: 263-4. (in Russian)
2. Volkov AV, Aleshkin VA. Vaccine for immunoprophylaxis and immunotherapy of human and animal diseases caused by pathogenic and conditionally pathogenic Gram-negative microorganisms of the intestinal group and their exotoxins, and the method of its preparation (variants), immunoglobulin preparation (variants) and the method of its preparation, immunobiological preparation, multicomponent vaccine. Patent RU 2 262 350. Published: 2005.10.20. (in Russian)
3. Lakhtin MV, Lakhtin VM, Aleshkin VA, Afanasiev SS. NK cells that recognize glycopatterns against tumors against the background of epidemiologically significant viral infections. *Health risk analysis* (Russia), 2019; 1: 144-53. ISSN (Print) 2308-1155. ISSN (Online) 2308-1163. ISSN (Eng-online) 2542-2308. (in Russian)
4. Lakhtin MV, Lakhtin VM, Aleshkin VA, Afanasiev SS. Glycoconjugate-recognizing proteins of human mucosal immunity: lectin systems of probiotic compartment of biotopes of mucous open cavities of the body. *Pharmacy* (Sankt-Peterburg), 2020; 69(1): 10-6. https://doi.org/10.29296/25419218-2020-01-02 (in Russian)
5. Lakhtin VM, Lakhtin MV, Davydkin VY. Glycoconjugate-recognizing systems in medical biotechnology. *Problems of scientific thought* (Ukraine), 2020; 3(6): 15-35.
6. Lakhtin MV, Lakhtin VM, Davydkin VY, Melikhova AV, Davydkin IY, Aleshkin VA. Postbiotics against infections, diseases and other pathologies. *Problems of scientific thought* (Ukraine), 2020; 3(7): 3-21. (in Russian)

7. Lakhtin VM, Lakhtin MV, Davydkin VY, Melikhova AV, Davydkin IY. Prospects of protective metabolic-cellular systems recognizing glycoconjugates in the prevention and therapy of covid infections and diseases. *News of GGTU. Medicine, Pharmacy* (Moscow), 2020; 4: 161-3. (in Russian) https://www.elibrary.ru/contents.asp?id=44583992 ; https://www.elibrary.ru/item.asp?id=44584053
8. Lakhtin VM, Lakhtin MV, Kombarova SY. COVID-19: Multi-target infection, protection strategies. *National priorities of Russia*, 2021; 3(42): 310-3. In Russian http://oniipi.org/материалы-всероссийской-научно-прак/
9. Lakhtin MV, Lakhtin VM, Mironov AY, Aleshkin VA, Afanasyev SS. Human pectin supersystems with probiotic and protective action. *Clinical Laboratory Diagnostics (Moscow),* 2020; 65(4): 231-8. doi:http://dx.doi.org/10.18821/0869-2020-65-4-231-238. (in Russian)
10. Ostanin AA, Kirikovich CC, Dolgova EV, Proskurina AS, Chernykh EP, Bogachev CC. The thorny path of macrophage-activating factor (GcMAF): from discovery to clinical practice. *Vavilov Journal of Genetics and Breeding*, 2019; 23(5): 624-31. DOI 10.18699/VJ19.535 (in Russian)
11. Sakhno LV, Shevela EY, Chernykh EP. Phenotypic and functional features of alternatively activated macrophages: possible use in regenerative medicine. *Immunology* (Moscow), 2015; 36(4): 242–6. (in Russian)
12. Tokayev EC, Rrasnova IS, Korobeynikova TV. Composition and clinical use of cow colostrum. *Nutrition issues* (Moscow), 2012; 81(3): 35-40. (in Russian)
13. Abbas S, Keir AK, Makrides M, Klein LD, Grzeskowiak LE, McPhee AJ, Rumbold AR. Tailoring Human Milk Oligosaccharides to Prevent Necrotising Enterocolitis Among Preterm Infants. *Frontiers in Nutrition*, Jul. 29, 2021; 8: 702888. doi: 10.3389/fnut.2021.702888.
14. Adams A, Sawh F, Green-Johnson JM, Taggart HJ, Strap JL. Characterization of casein-derived peptide bioactivity: Differential effects on angiotensin-converting enzyme inhibition and cytokine and nitric oxide production. *Journal of Dairy Science*, 2020; 103(7): 5805-15. doi: 10.3168/jds.2019-17976.
15. Ahtesh FB, Stojanovska L, Apostolopoulos V. Anti-hypertensive peptides released from milk proteins by probiotics. *Maturitas,* 2018; 115: 103-9. doi: 10.1016/j.maturitas.2018.06.016.
16. Arpitha A, Santhosh MS, Rohit AC, Girish KS, Vinod D, Aparna HS. Inhibition of Snake Venom Metalloproteinase by beta-Lactoglobulin Peptide from Buffalo (*Bubalus bubalis*)

Colostrum. *Applied Biochemistry and Biotechnology*, 2017; 182(4): 1415-32. doi: 10.1007/s12010-017-2407-6.

17. Arslan A, Kaplan M, Duman H, Bayraktar A, Ertürk M, Henrick BM, Frese SA, Karav S. Bovine Colostrum and Its Potential for Human Health and Nutrition. *Frontiers in Nutrition,* Jun. 21, 2021; 8: 651721. doi: 10.3389/fnut.2021.651721.
18. Autran CA, Schoterman MHC, Jantscher-Krenn E, Kamerling JP, Bode L. Sialylated galacto-oligosaccharides and 2'-fucosyllactose reduce necrotising enterocolitis in neonatal rats. *Br J Nutr,* 2016; 116(2): 294-9. doi: 10.1017/S0007114516002038.
19. Bakunina I, Chadova O, Malyarenko O, Ermakova E. The Effect of Fucoidan from the Brown Alga *Fucus evanescence* on the Activity of alpha-N-Acetylgalactosaminidase of Human Colon Carcinoma Cells. *Marine Drugs,* 2018; 16: 155. doi:10.3390/md16050155 www.mdpi.com/journal/marinedrugs
20. Bello M. Structural and energetic requirements for a second binding site at the dimeric beta-lactoglobulin interface. *Journal of Biomolecular Structure and Dynamics*, 2016; 34(9): 1-54. doi: 10.1080/07391102.2015.1094413.
21. Birch J, Khan S, Madsen M, Kjeldsen C, Møller SM, Stender EGP, Peters GHJ, Duus JØ, Kragelund BB, Svensson B. Binding Sites for Oligosaccharide Repeats from Lactic Acid Bacteria Exopolysaccharides on Bovine beta-Lactoglobulin Identified by NMR Spectroscopy. *ACS Omega*, 2021; 6(13): 9039-52. doi: 10.1021/acsomega.1c00060.
22. Brink LR, Chichlowski M, Pastor N, Narayanappa AT, Shah N. In the Age of Viral Pandemic, Can Ingredients Inspired by Human Milk and Infant Nutrition Be Repurposed to Support the Immune System? *Nutrients*, Mar 6, 2021; 13(3): 870. doi: 10.3390/nu13030870.
23. Cabral H. Peptides: Molecular and Biotechnological Aspects. *Biomolecules,* Jan 1, 2021; 11(1): 52. doi: 10.3390/biom11010052.
24. Cao C, Song D, Yang M, Yang N, Ye Q, Tao D, Liu B, Wu R, Yue R. Comparative Analysis of Whey N-Glycoproteins in Human Colostrum and Mature Milk Using Quantitative Glycoproteomics. *Journal of Agricultural and Food Chemistry,* 2017; 65(47): 10360-7. doi: 10.1021/acs.jafc.7b04381
25. Cao X, Yang M, Yang N, Liang X, Tao D, Liu B, Wu J, Yue X. Characterization and comparison of whey N-glycoproteomes from human and bovine colostrum and mature milk. *Food Chemistry,* 2019; 276: 266-73. doi: 10.1016/j.foodchem.2018.09.174.
26. Chandwe K, Kelly P. Colostrum Therapy for Human Gastrointestinal Health and Disease. *Nutrients,* Jun. 7, 2021; 13(6): 1956. doi: 10.3390/nu13061956.

27. Chougule RA, Aparna HS. Characterization of beta-lactoglobulin from buffalo (*Bubalus bubalis*) colostrum and its possible interaction with erythrocyte lipocalin-interacting membrane receptor. *Journal of Biochemistry,* 2011; 150(3): 279-88. doi: 10.1093/jb/mvr054.
28. Chougule RA, Shilpa P, Salimath BP, Sosalegowda AH. Buffalo colostrum beta-lactoglobulin inhibits VEGF-induced angiogenesis by interacting with G protein-coupled receptor kinase. *Applied Biochemistry and Biotechnology*. 2013; 171(2): 366-81. doi: 10.1007/s12010-013-0344-6.
29. Comstock SS, Li M, Wang M, Monaco MH, Kuhlenschmidt TB, Kuhlenschmidt MS, Donovan SM. Dietary Human Milk Oligosaccharides but Not Prebiotic Oligosaccharides Increase Circulating Natural Killer Cell and Mesenteric Lymph Node Memory T Cell Populations in Noninfected and Rotavirus-Infected Neonatal Piglets. *Journal of Nutrition*. 2017; 147(6): 1041-7. doi: 10.3945/jn.116.243774.
30. Davison G. The Use of Bovine Colostrum in Sport and Exercise. *Nutrients,* May 24 2021; 13(6): 1789. doi: 10.3390/nu13061789.
31. Delanghe JR, Speeckaert R, Speeckaert MM. Behind the scenes of vitamin D binding protein: more than vitamin D binding. *Best Pract Res Clinical Endocrinology and Metabolism,* 2015; 29(5): 773-86. doi: 10.1016/j.beem.2015.06.006.
32. Domínguez-Ramírez L, Del Moral-Ramírez E, Cortes-Hernández P, García-Garibay M, Jiménez-Guzmán J. beta-Lactoglobulin's conformational requirements for ligand binding at the calyx and the dimer interphase: a flexible docking study. *PLoS One,* Nov 8, 2013; 8(11): e79530. doi: 10.1371/journal.pone.0079530.
33. Donovan SM, Comstock SS. Human Milk Oligosaccharides Influence Neonatal Mucosal and Systemic Immunity. *Annals of Nutrition and Metabolism,* 2016; 69(2): 42-51. doi: 10.1159/000452818.
34. Gobbetti M., Stepaniak L., De Angelis M., Corsetti A., Di Cagno R. Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing. *Critical Revirws in Food Scitnce and Nutrition,* 2002; 42(3): 223-39. doi: 10.1080/10408690290825538.
35. Gonzalez-Gonzalez C, Gibson T, Jauregi P. Novel probiotic-fermented milk with angiotensin I- converting enzyme inhibitory peptides produced by *Bifidobacterium bifidum* MF 20/5. *International Journal of Food Microbiology,* 2013; 167(2): 131-7. doi: 10.1016/j.ijfoodmicro.2013.09.002.
36. Hernández-Ledesma B, Recio I, Amigo L. beta-Lactoglobulin as source of bioactive

peptides. *Amino Acids,* 2008; 35(2): 257-65. doi: 10.1007/s00726-007-0585-1.

37. Hughes A, Brock JH, Parrott DM, Cockburn F. Effect of human colostrum and infant formula on the phagocytic activity of macrophages. I. Resident and stimulated mouse peritoneal macrophages. *Clinical and Experimental Immunology,* 1985; 61(1): 169-75.
38. Hung Y-P, Lee C-C, Lee J-C, Tsai P-J, Ko W-C. Gut Dysbiosis during COVID-19 and Potential Effect of Probiotics. *Microorganisms,* Jul. 28, 2021; 9(8): 1605. doi: 10.3390/microorganisms9081605.
39. Ichihashi M, Nakamura Y, Muto M, Nishikata T, Inui T, Uto Y. A case of chronic actinic dermatitis that responded completely to treatment with oral colostrum-macrophage-activating factor (colostrum-MAF). *Photodermatology Photoimmunology and Photomedicine,* 2019; 35(4): 290-2. doi: 10.1111/phpp.12469.
40. Inui T, Kubo K, Kuchiike D, Uto Y, Nishikata T, Sakamoto N, Mette M. Oral Colostrum Macrophage-activating Factor for Serious Infection and Chronic Fatigue Syndrome: Three Case Reports. *Anticancer Research,* 2015; 35(8): 4545-9.
41. Italiani P, Camera GD, Boraschi D. Induction of Innate Immune Memory by Engineered Nanoparticles in Monocytes/ Macrophages: From Hypothesis to Reality. *Frontiers in Immunology,* Oct. 6, 2020; 11: 566309. doi: 10.3389/fimmu.2020.566309.
42. Jenny M, Pedersen NR, Hidayat BJ, Schennach H, Fuchs D. Bovine colostrum modulates immune activation cascades in human peripheral blood mononuclear cells *in vitro*. *New Microbiologica,* 2010; 33(2): 129-35.
43. Ju T, Otto VI, Cummings RD. The Tn Antigen—Structural Simplicity and Biological Complexity. *Angewandte Chemie International Edition English,* 2011; 50(8): 1770–91. doi: 10.1002/anie.201002313
44. Kazimierska K, Kalinowska-Lis U. Milk Proteins - Their Biological Activities and Use in Cosmetics and Dermatology. *Molecules,* May 28, 2021; 26(11): 3253. doi: 10.3390/molecules26113253.
45. Khan S, Ipsen R, Almdal K, Svensson B, Harris P. Revealing the Dimeric Crystal and Solution Structure of beta-Lactoglobulin at pH 4 and Its pH and Salt Dependent Monomer-Dimer Equilibrium. *Biomacromolecules,* 2018; 19(7): 2905-12. doi: 10.1021/acs.biomac.8b00471.
46. Kontopidis G, Holt C, Sawyer L. Invited review: beta-lactoglobulin: binding properties, structure, and function. *Journal of Dairy Science,* 2004; 87(4): 785-96. doi: 10.3168/jds.S0022-0302(04)73222-1.
47. Korhonen HJ. Production and properties of health-promoting proteins and peptides from

bovine colostrum and milk. *Cellular and Molecular Biology (Noisy-le-grand, France)*, 2013; 59(1): 12-24.

48. Kurian SJ, Unnikrishnan MK, Miraj SS, Bagchi D, Banerjee M, Reddy BS, Rodrigues GS, Manu MK, Saravu K, Mukhopadhyay C, Rao M. Probiotics in Prevention and Treatment of COVID-19: Current Perspective and Future Prospects. *Archives of Medical Research*, 2021; 52(6): 582-94. doi: 10.1016/j.arcmed.2021.03.002.
49. Law BA, Kolstad J. Proteolytic systems in lactic acid bacteria. *Antonie Van Leeuwenhoek*, 1983; 49(3): 225-45. doi: 10.1007/BF00399500.
50. Lakhtin M, Alyoshkin V, Lakhtin V, Afanasyev S, Pozhalostina L, Pospelova V. Probiotic lactobacillus and bifidobacterial lectins against *Candida albicans* and *Staphylococcus aureus* clinical strains: new class of pathogen biofilm destructors. *Probiotics and Antimicrobial Proteins*, 2010; 2: 186-96, doi:10.1007/s12602-010-9046-3.
51. Lakhtin M, Lakhtin V, Aleshkin A, Bajrakova A, Afanasiev S, Aleshkin V. Lectin systems imitating probiotics: potential for biotechnology and medical microbiology // In: “Probiotics 2012”, Edited by E.C. Rigobelo. – New York, InTech, 2012; 417–432. ISBN 978-953-51-0776-7.
52. Lakhtin M, Lakhtin V, Afanasiev S, Aleshkin V. Probiotic lectins communicate to innate autoimmunity. *Proceedings of the 11th Autoimmunity*. 2018. All abstracts: abs AUTO1-0653 (electronic page 775). http://www.autoimmunity-network.com/media/moxie/files/a/ad/adm/admin/AUTO%202018%20-%20abstracts%20for%20USB.pdf
53. Lakhtin VM, Lakhtin MV, Davydkin VY, Melikhova AV, Davydkin IY, Zhilenkova OG. Enzybiotics with lectin properties and lectinbiotics with enzyme activities - multifunctional modulators of communications in organism: fundamentals and unlimited potential in food industry, biosensors and medicine. *EJBIO*, 2021; 2(1): 38-46. DOI: http://dx.doi.org/10.24018/ejbio.2021.2.1.150
54. Manzanarez-Quín CG, Beltrán-Barrientos LM, Hernández-Mendoza A, González-Córdova AF, Vallejo-Cordoba B. Invited review: Potential antiobesity effect of fermented dairy products. *Journal of Dairy Science*, 2021; 104(4): 3766-78. doi: 10.3168/jds.2020-19256.
55. Marranzino G, Villena J, Salva S, Alvarez S. Stimulation of macrophages by immunobiotic *Lactobacillus* strains: influence beyond the intestinal tract. *Microbiology and Immunology*, 2012; 56(11): 771-81. doi: 10.1111/j.1348-0421.2012.00495.x.
56. Nabeshima Y, Abe C, Kawauchi T, Hiroi T, Uto Y, Nabeshima Y-I. Simple method for

large-scale production of macrophage activating factor GcMAF. *Scientific Reports,* 2020; 10: 19122. doi: 10.1038/s41598-020-75571-y

57. Nagpal R, Behare P, Rana R, Kumar A, Kumar M, Arora S, Morotta F, Jain S, Yadav H. Bioactive peptides derived from milk proteins and their health beneficial potentials: an update. *Food and Function,* 2011; 2(1): 18-27. doi: 10.1039/c0fo00016g.
58. Nanjundaiah YS, Wright DA, Baydoun AR, Khaled Z, Ali Z, Dean P, Sarker MH. Modulation of Macrophage Function by *Lactobacillus*-Conditioned Medium. *Frontiers in Cell and Developmental Biology,* Jul 30, 2020; 8: 723. doi: 10.3389/fcell.2020.00723.
59. Orczyk-Pawiłowicz M, Lis-Kuberka J. The Impact of Dietary Fucosylated Oligosaccharides and Glycoproteins of Human Milk on Infant Well-Being. *Nutrients,* Apr. 16, 2020; 12(4): 1105. doi: 10.3390/nu12041105.
60. Orth K, Knoefel WT, van Griensven M, Matuschek C, Peiper M, Schrumpf H, Gerber PA, Budach W, Bölke E, Buhren BA, Schauer M. Preventively enteral application of immunoglobulin enriched colostrums milk can modulate postoperative inflammatory response. *European Journal of Medical Research,* Nov. 23, 2013; 18(1): 50. doi: 10.1186/2047-783X-18-50.
61. Pace RM, Williams JE, Järvinen KM, Belfort MB, Pace CDW, Lackey KA, Gogel AC, Nguyen-Contant P, Kanagaiah P, Fitzgerald T, Ferri R, Young B, Rosen-Carole C, Diaz N, Meehan CL, Caffé B, Sangster MY, Topham D, McGuire MA, Seppo A, McGuire MK. Characterization of SARS-CoV-2 RNA, Antibodies, and Neutralizing Capacity in Milk Produced by Women with COVID-19. *mBio,* Feb. 9, 2021; 12(1): e03192-20. doi: 10.1128/mBio.03192-20.
62. Pellon A, Barriales D, Peña-Cearra A, Castelo-Careaga J, Palacios A, Lopez N, Atondo E, Pascual MA. The commensal bacterium *Lactiplantibacillus plantarum* imprints innate memory-like responses in mononuclear phagocytes. *Gut Microbes,* Jan-Dec, 2021; 13(1): 1939598. doi: 10.1080/19490976.2021.1939598.
63. Playford RJ, Weiser MJ. Colostrum: Its Constituents and Uses. *Nutrients,* Jan. 18, 2021; 13(1): 265. doi: 10.3390/nu13010265.
64. Playford RJ, Weiser MJ, Marchbank T. Methods to improve efficacy of orally administered bioactive peptides using bovine colostrum as an exemplar. *PLoS One,* Jun. 17, 2021; 16(6): e0253422. doi: 10.1371/journal.pone.0253422.
65. Rasmussen SO, Martin L, Østergaard MV, Rudloff S, Li Y, Roggenbuck M, Bering SB, Sangild PT. Bovine colostrum improves neonatal growth, digestive function, and gut immunity relative to donor human milk and infant formula in preterm pigs. *American*

Journal of Physiology, Gastrointestinal and Liver Physiology, 2016; 311(3): G480-91. doi: 10.1152/ajpgi.00139.2016.

66. Rohit AC, Sathisha K, Aparna HS. A variant peptide of buffalo colostrum beta-lactoglobulin inhibits angiotensin I-converting enzyme activity. *European Journal of Medical Chemistry,* 2012; 53: 211-9. doi: 10.1016/j.ejmech.2012.03.057.
67. Rubak YT, Nuraida L, Iswantini D, Prangdimurti E. Angiotensin-I-converting enzyme inhibitory peptides in milk fermented by indigenous lactic acid bacteria. *Veterinary World,* 2020; 13(2): 345-53. doi: 10.14202/vetworld.2020.345-353.
68. Saburi E, Saburi A, Ghanei M. Promising role for Gc-MAF in cancer immunotherapy: from bench to bedside. *Caspian Journal of Internal Medicine,* 2017; 8(4): 228-38. doi: 10.22088/cjim.8.4.228.
69. Sangild PT, Vonderohe C, Hebib VM, Burrin DG. Potential Benefits of Bovine Colostrum in Pediatric Nutrition and Health. *Nutrients*, Jul. 26, 2021; 13(8): 2551. doi: 10.3390/nu13082551.
70. Sawyer L. beta-Lactoglobulin and Glycodelin: Two Sides of the Same Coin? *Frontiers in Physiology,* May 20, 2021; 12: 678080. doi: 10.3389/fphys.2021.678080.
71. Sienkiewicz M, Szymańska P, Fichna J. Supplementation of Bovine Colostrum in Inflammatory Bowel Disease: Benefits and Contraindications. *Advances in Nutrition,* 2021; 12(2): 533-45. doi: 10.1093/advances/nmaa120.
72. Skrzypczak K, Fornal E, Domagała D, Gustaw W, Jabłońska-Ryś E, Sławińska A, Radzki W, Kononiuk A, Waśko A. Use of alpha-Lactalbumin and Caseinoglycomacropeptide as Biopeptide Precursors and as Functional Additives in Milk Beverages Fermented by *L. helveticus*. *International Journal of Food Science,* Apr. 13, 2021; 2021: 8822161. doi: 10.1155/2021/8822161.
73. Soboleva SE, Zakharova OD, Sedykh SE, Alinovskaya.LI, Buneva VN, Nevinsky GA. Cow Milk Lactoferrin Possesses Several Catalytic Activities. *Biomolecules*, May 29, 2019; 9(6): 208. doi: 10.3390/biom9060208.
74. Spadera L, Spadera M. Potential role of GcMAF in suppressing the severity of COVID-19-induced immune responses: Lesson learned from HIV. *Medical Hypotheses,* Nov, 2020; 144: 110293. doi: 10.1016/j.mehy.2020.110293.
75. Speeckaert MM, Delanghe JR. The potential significance of vitamin D binding protein polymorphism in COVID-19. *International Journal of Infectious Diseases,* Aug. 2021; 109: 90. doi: 10.1016/j.ijid.2021.06.044
76. Speeckaert MM, Speeckaert R, Delanghe JR. Vitamin D and Vitamin D binding protein:

the inseparable duo in COVID-19. *Journal of Endocrinological Investigation*, 2021; 44(10): 2323-4. doi: 10.1007/s40618-021-01573-w.

77. Stender EGP, Birch J, Kjeldsen C, Nielsen LD, Duus JØ, Kragelund BB, Svensson B. Alginate Trisaccharide Binding Sites on the Surface of beta-Lactoglobulin Identified by NMR Spectroscopy: Implications for Molecular Network Formation. *ACS Omega*, 2019; 4(4): 6165-74. doi: 10.1021/acsomega.8b03532
78. Struff WG, Sprotte G. Bovine colostrum as a biologic in clinical medicine: a review--Part II: clinical studies. *International Journal of Clinical Pharmacolology and Therapeutics*, 2008; 46(5): 211-25. doi: 10.5414/cpp46211.
79. Sultan S, Huma N, Butt MS, Aleem M, Abbas M. Therapeutic potential of dairy bioactive peptides: A contemporary perspective. *Critical Reviews in Food Science and Nutrition*, 2018; 58(1): 105-15. doi: 10.1080/10408398.2015.1136590.
80. Sun J, Li Y, Pan X, Nguyen DN, Brunse A, Bojesen A, Rudloff S, Mortensen MS, Burrin D, Sangild P. Human Milk Fortification with Bovine Colostrum Is Superior to Formula-Based Fortifiers to Prevent Gut Dysfunction, Necrotizing Enterocolitis, and Systemic Infection in Preterm Pigs. *Journal of Parenteral and Enteral Nutrition*, 2019; 43(2): 252-62. doi: 10.1002/jpen.1422.
81. Uto Y, Kawai T, Sasaki T, Hamada K, Yamada H, Kuchiike D, Kubo K, Inui T, Mette M, Tokunaga K, Hayakawa A, Go A, OOsaki T. Degalactosylated/ Desialylated Bovine Colostrum Induces Macrophage Phagocytic Activity Independently of Inflammatory Cytokine Production. *Anticancer Research*, 2015; 35(8): 4487-92.
82. Vassilopoulou E, Feketea G, Koumbi L, Mesiari C, Berghea EC, Konstantinou GN. Breastfeeding and COVID-19: From Nutrition to Immunity. *Frontiers in Immunology*, Apr. 7, 2021; 12: 661806. doi: 10.3389/fimmu.2021.661806.
83. Wei H, Xu Y, Cheng B, Xiong Y. Synergistic effects of *Lactobacillus rhamnosus* ZDY114 and bovine colostrums on the immunological function of mouse *in vivo* and *in vitro*. *Applied Microbiology and Biotechnology*, 2007; 75(2): 427-34. doi:10.1007/s00253-006-0818-3.
84. Widyastuti Y, Febrisiantosa A, Tidona F. Promoting Properties of Lactobacilli in Fermented Dairy Products. *Frontiers in Microbiology*, May 21, 2021; 12: 673890. doi: 10.3389/fmicb.2021.673890.
85. Yamamoto N, Takano T. Antihypertensive peptides derived from milk proteins. *Nahrung*, 1999; 43(3): 159-64. doi: 10.1002/(SICI)1521-3803(19990601)43:3<159::AID-FOOD159>3.0.CO;2-R.

86. Yang M-C, Guan H-H, Liu M-Y, Lin Y-H, Yang J-M, Chen W-L. Crystal structure of a secondary vitamin D3 binding site of milk beta-lactoglobulin. *Proteins*, 2008; 71(3): 1197-210. doi: 10.1002/prot.21811.
87. Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W. Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Frontiers in Bioengineering and Biotechnology*, May. 12, 2021; 9: 612285. doi: 10.3389/fbioe.2021.612285.