Evaluation of the biological activity of the GM-CSF-ApoA-I fusion protein, obtained by biosynthesis in the yeast Pichia pastoris

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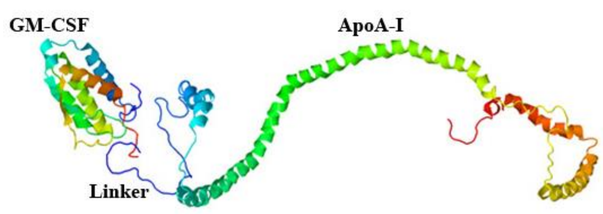
INTRODUCTION

Clinicians use recombinant GM-CSF to treat neutropenia and reduce the risk of infections during bone marrow transplantation, but the short half-life and high toxicity of GM-CSF are serious limitations of its use in therapy. Two strategies are mainly used - pegylation of GM-CSF and the construction of fusion proteins to increase the clinical efficacy of GM-CSF. In this study a chimera was created in which GM-CSF was fused with apolipoprotein A-I (apoA-I) to reduce the toxicity of GM-CSF. ApoA-I is a naturally occurring protein with a long half-life in the body with atheroprotective[1], antioxidant[2], antiapoptotic[3] and anti-inflammatory[4] effects.



Representation of the chimeric protein ryGM-ApoA-I tertiary structure.

Results and discussion

GM-CSF-ApoA-I was expressed in methylotrophic yeast *Pichia pastoris*.
Preparative production of the recombinant yeast-derived GM-CSF-ApoA-I (ryGM-CSF-ApoA-I) was carried out by cultivating the yeast cells in conical flasks on an orbital shaker in the presence of 1.0% methanol. The yield of GM-CSF-ApoA-I was 60 mg/L.
The chimera was purified by successive chromatography on DEAE- and SP Sepharose FF to 95% purity.

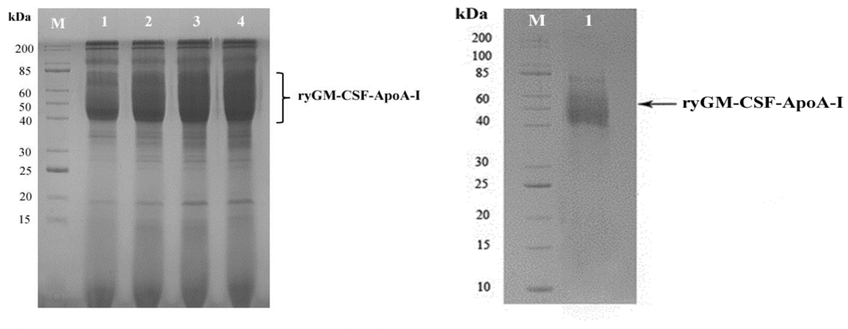


Fig.1. Production and purification ryG-CMSF-ApoA-I.
(A) - Production protein in a shake-flask. Lane M: protein molecular weight marker (Sib Enzyme); lanes 1–4 Induction of protein synthesis by 24, 48, 72, 96 h.
(B) - SDS-PAGE analysis of purified ryGM-CSF-ApoA-I preparations.

Analysis of biological activity ryGM-CSF-ApoA-I

Myeloid stimulating activity on human bone marrow cells

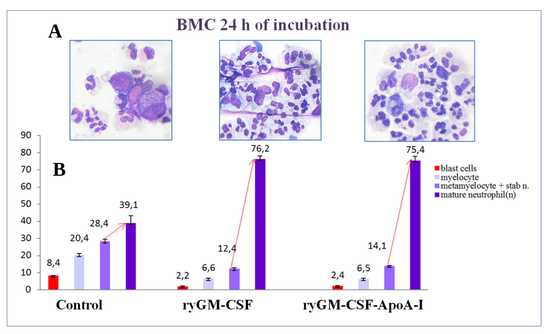


Fig.2. Stimulation of the maturation of cells of the granulocyte series by ryGM-CSF and ryGM-CSF –ApoA-I. (A) - Representative picture of segmented neutrophils obtained under the influence of growth factors
(B) - Distribution of granulocytic cells according to the degree of their maturation in the presence of ryGM-CSF and ryGM-CSF-ApoA-I.

RyGM-CSF-ApoA-I stimulated granulopoiesis comparable to authentic cytokine.

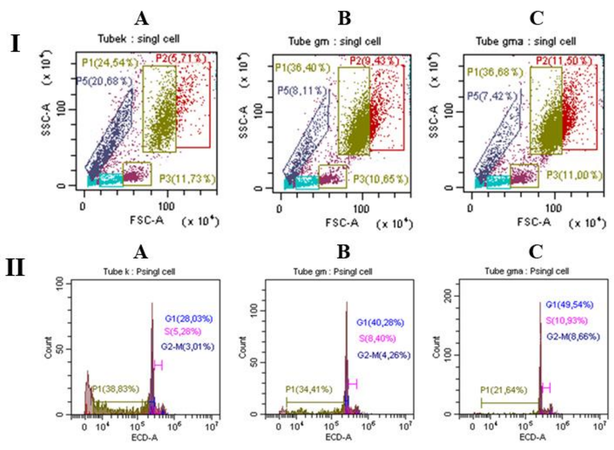


Fig.3. Flow cytometric analysis of all BMCs.
(I) - Living cells under the influence of growth factors after 48 h of incubation.
Gate P1 - mature segmented neutrophils. In the case of ryGM-CSF – 36,4%, ryGM-CSF-ApoA-I – 36,7%. Gate P2 - granulocytic proliferating cells -ryGM-CSF- 9,43%, ryGM-CSF-ApoA-I - 11,5%.
(II) - Cell cycle of all BMCs. P1 gate contains apoptotic cells (ryGM-CSF–34,4%, ryGM-CSF–ApoA-I-21,6%. Cells in the active cycle S+G2/M (ryGM-CSF–12,6%; ryGM-CSF–ApoA-I–19,6%).

RyGM-CSF-ApoA-I maintained proliferation, decreased apoptotic cell death, and retained the pool of granulocyte progenitor BMCs more efficiently than ryGM-CSF[5].

Stimulation of the generation of dendritic cells

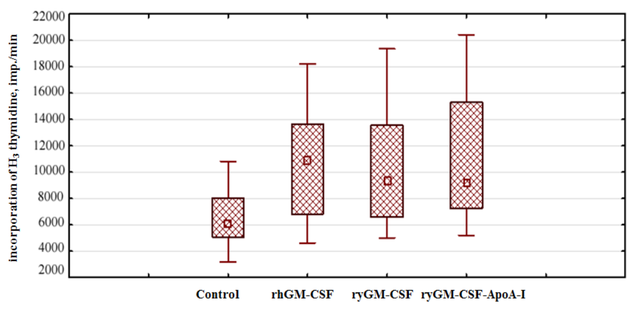


Fig.4.Allostimulatory activity of dendritic cells (DC) generated in the presence of various GM-CSFs. The control differed significantly from all tested growth factors ($p < 0.005$).

The biological activity of cytokines was assessed by the ability to induce differentiation of monocytes in DC, in the presence of growth factors 40 ng/ml GM-CSF and 1000 U/ml IFN-α, followed by maturation in the presence of lipopolysaccharide. Blood mononuclear cells were obtained with the voluntary consent of 10 individuals.
The efficiency of DC induction was monitored by the expression of markers of monocytes (CD14), co-stimulatory (CD86), and mature DCs (CD83). The proliferation of allogeneic lymphocytes stimulated by DC was assessed by the incorporation of H3 thymidine.

DCs obtained by incubation with ryGM-CSF-ApoA-I have a similar effect to commercial GM-CSF (Sigma-Aldrich) and ryGM-CSF. There was no statistically significant difference between the 3 growth factors in the ability to stimulate lymphocyte proliferation.

Conclusion

RyGM-CSF–ApoA-I retained the ability of GM-CSF to stimulate proliferation and maturation of bone marrow granulocytes, as well as differentiation and maturation of DCs from human mononuclear cells. The chimeric form more effectively maintains the viability of BMCs by decreasing apoptosis and maintaining the proliferative potential of cells for a longer time.

References

1. Morton J., Bao S., Vanags L.Z., Tsatralis T., Ridiandries A., Siu C.W., Ng K.M., Tan JT.M., Celermajer D.S., Ng MK.C., Bursill C.A. Strikingly Different Atheroprotective Effects of Apolipoprotein A-I in Early- Versus Late-Stage Atherosclerosis. *JACC Basic. Transl. Sci.* **2018,** 3, 187-199.
2. Garner B., Waldeck A.R., Witting P.K., Rye K.A., Stocker R. Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. *J. Biol. Chem.* **1998**, 273, 6088–6095.
3. Suc I., Escargueil-Blanc I., Troly M., Salvayre R., Negre-Salvayre A. HDL and apoA prevent cell death of endothelial cells induced by oxidized LDL. *Arterioscler. Thromb. Vasc. Biol*. **1997**, 17, 2158– 2166.
4. Hyka N., Dayer J.M., Modoux C., Kohno T., Edwards C.K., Roux-Lombard P., Burger D. Apolipoprotein A-I inhibits the production of interleukin-1 and tumor necrosis factor- by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood*, **2001**, 97, 2381–2389.
5. Pykhtina M., Miroshnichenko S., Romanov V., Grazhdantseva A., Kochneva G., Beklemishev A. Construction of Recombinant Human GM-CSF and GM-CSF-ApoA-I Fusion Protein and Evaluation of Their Biological Activity. *Pharmaceuticals (Basel)*. **2021,** 14(5):459.