Erythropoietin receptor is detectable on peripheral blood lymphocytes and its expression increases in activated T lymphocytes

We are writing to comment on the article by Lifshitz L *et al.* published in Haematologica in June 2010: "Macrophages as novel target cells for erythropoietin".[1] We read the article with great interest but we have to stress that the Authors have passed over our publications concerning immunomodulatory action of recombinant human erythropoietin (rhEPO) from over the last 12 years. Thus, based on our own observations which were published before the Lifshitz paper, we have to challenge their claims that "….EPO-receptors were not detectable in lymphocytes…" (Introduction, p. 1,824 supported by citation of Prutchi-Sagiv *et al.*[2]) and "however, EPO-receptor expression or direct EPO stimulation on lymphocytes could not be demonstrated to date".

Recombinant human erythropoietin is commonly used for treatment of anemia related to chronic renal failure (CRF). Receptor for erythropoietin (EPO-R) has been found in other tissues and cells apart from erythroid cells, including neurons, endothelial cells, some solid tumor cells and various types of renal cells, but also polymorphonuclear leukocytes.[3] Both EPO-R structure and its presence on leukocytes indicated that beyond erythropoietic function, EPO might possess some immunomodulatory properties. Patients who have undergone hemodialysis (HD) exhibit deficiency in both cell mediated and humoral immunity and that is why they presented good material for studies concerning rhEPO. We have demonstrated a direct influence of rhEPO treatment on secretion of cytokines (IL-2, IL-10 and TNF-α) for the first time in 1998[4,5] and on activation parameters of $CD4^+$ T lymphocytes[6] in HD patients with chronic renal failure (CRF). We found that addition of rhEPO *in vitro* to the whole blood cell cultures of the HD patients before implementation of rhEPO directly stimulates IL-2 production. Also rhEPO therapy itself affected IL-2 production.[4] In the meantime, level of IL-10 increased in all rhEPO-treated patients during the therapy, while level of TNF-α was temporarily decreased.[5] RhEPO treatment normalized the decreased expression of CD28 and CD69 on $CD4^+$ lymphocytes of HD patients. We have also studied the kinetics of proliferation of $CD4^+$ T lymphocytes by DCT cytometric technique and we found that fewer $CD4^+CD28^+$ T cells of patients not receiving rhEPO proliferated after stimulation; these cells needed longer to enter the first division cycle.[6]

Moreover, this year we have also published an article that for the first time describes presence of erythropoietin receptor (EPO-R) on human blood lymphocyte and monocyte surface using quantitative flow cytometry to calculate the exact numbers of these receptors.[7] The notion of EPO-R being expressed by human lymphocytes was supported by our finding of EPO-R mRNA in peripheral blood mononuclear cells as well as in isolated $CD4^+$ T lymphocytes. The results suggested that rhEPO action on those cells can be direct.

To verify this hypothesis, we have stimulated peripheral blood mononuclear cells (PBMC) with immobilized anti-CD3 antibody (250 ng per 2 million cells in 2 mL of complete medium) and incubated for five days at 37°C, 5% CO_2. Stimulated cells were collected after 48, 72 and 120 h and stained with PE-conjugated anti-EpoR monoclonal antibody and RPE-Cy5-conjugated anti-CD4 for 30 min at 4°C. After washing twice in PBS, cells were resuspended in 200 μL of the same buffer for flow cytometric analysis performed directly after sample preparation. Quantitative fluorescence analysis assessing the numbers of EPO-R bound per cell was performed using Phycoerythrin Fluorescence Quantitation Kit (Becton Dickinson, USA) according to the manufacturer's protocol, and a FACScan flow cytometer (Becton Dickinson, USA).[7] Stimulation of PBMC *in vitro* resulted in increased EPO-R molecule number on $CD4^+$ lymphocytes after 48

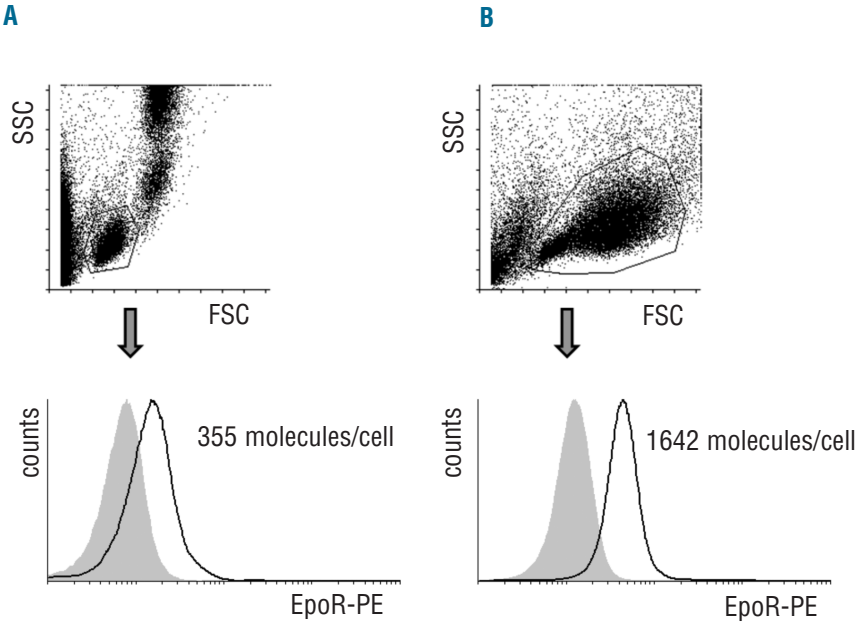

Figure 1. EPO-R expression on $CD4^+$ T lymphocytes *ex vivo* (A) and $CD4^+$ and $CD4^+$ T lymphocytes stimulated with immobilized anti-CD3 antibody. (B) Figure shows representative 2 flow cytometry histograms for 30 experiments yielding similar results. EPO-R expression (black line) was estimated as a mean fluorescence shift toward control unstained with anti-EpoR monoclonal antibody (gray field), $P<0.05$, Kolmogorov-Smirnov test. Number of EPO-R was calculated using Phycoerythrin Fluorescence Quantitation Kit (Becton Dickinson, USA) according to the manufacturer's protocol.

h of stimulation.[7] Representative histograms are presented in Figure 1. A detailed analysis of EPO-R molecules per one cell is described in our article.[7]

We would like to emphasize once more that our studies from over the last 12 years demonstrated that erythropoietin is probably able to modulate or amplify some signaling pathways important for human lymphocyte and monocyte functions. There are also many studies, including ours, demonstrating the role of rhEPO in improving immune responses in CRF patients and at the same time suggesting that EPO/rhEPO may act as an immunomodulatory cytokine in the human organism.

Katarzyna A. Lisowska, Ewa Bryl, and Jacek M. Witkowski

Department of Pathophysiology, Medical University of Gdańsk, Poland

Correspondence: Lisowska Katarzyna, Department of Pathophysiology, Medical University of Gdańsk, Dębinki 7, 80-211 Gdańsk, Poland. Email: katlis@gumed.edu.pl

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References

1. Lifshitz L, Tabak G, Mittelman M, Gassmann M, Neumann D. Macrophages as novel targets for erythropoietin. Haematologica. 2010;95(11):1823-40.
2. Prutchi-Sagiv S, Golishevsky N, Oster HS, Katz O, Cohen A, Naparstek E, et al. Erythropoietin treatment in advanced multiple myeloma is associated with improved immunological functions: could it be beneficial in early disease? Br J Haematol. 2006;135(5):660-72.
3. Sela S, Shurtz-Swirski R, Sharon R, Manaster J, Chezar J, Shkolnik G, et al. The polymorphonuclear leukocyte--a new target for erythropoietin. Nephron. 2001;88(3):205-10.
4. Bryl E, Mysliwska J, Debska-Slizien A, Trzonkowski P, Rachon D, Bullo B, et al. Recombinant human erythropoietin stimulates production of interleukin 2 by whole blood cell cultures of hemodialysis patients. Artif Organs. 1999;23(9):809-16.
5. Bryl E, Mysliwska J, Debska-Slizien A, Rachon D, Bullo B, Lizakowski S, et al. The influence of recombinant human erythropoietin on tumor necrosis factor alpha and interleukin-10 production by whole blood cell cultures in hemodialysis patients. Artif Organs. 1998;22(3):177-81.
6. Lisowska KA, Debska-Slizien A, Radzka M, Witkowski JM, Rutkowski B, Bryl E. Recombinant human erythropoietin treatment of chronic renal failure patients normalizes altered phenotype and proliferation of CD4-positive T lymphocytes. Artif Organs. 2010;34(3):E77-84.
7. Lisowska KA, Debska-Slizien A, Bryl E, Rutkowski B, Witkowski JM. Erythropoietin receptor is expressed on human peripheral blood T and B lymphocytes and monocytes and is modulated by recombinant human erythropoietin treatment. Artif Organs. 2010;34(8):654-62.