Neocytolysis: Quantification, characterization, and mechanisms of the destruction of newly formed erythrocytes upon return from high altitude

Heimo Mairbäurl[1], Lars Kaestner[2], Anna Bogdanova[1]

[1] Medical Clinic VII, Sports Medicine, University Hospital Heidelberg, Heidelberg, Germany
(**new address**: Translational Pneumology, University Hospital Heidelberg, Heidelberg, Germany)
[2] Theoretical Medicine and Biosciences &Experimental Physics, Saarland University; Homburg / Saarbrücken, Germany
[3] Red Blood Cell Research Group, Institute of Veterinary Physiology, University of Zürich, Zürich, Switzerland

heimo.mairbaeurl@med.uni-heidelberg.de

Keywords: high altitude; erythropoiesis; neocytolysis; erythrocyte membrane biochemistry; erythroid precursor differentiation

1. Project description

1.1. Introduction: Exposure to high altitude stimulates erythropoiesis in lowlanders, which increases the total amount of hemoglobin in circulation in order to increase the oxygen binding capacity under conditions of limited oxygen loading of hemoglobin at high altitude, which is caused by the low oxygen partial pressure of the inspired air.

The total hemoglobin mass increases during a prolonged stay at high altitude, and it decreases rapidly, i.e., within one to two weeks, upon descent to low altitude. Thus, the number of circulating erythrocytes matches oxygen availability in both the hypoxic and the normoxic environment. There is some evidence from the literature indicating that the erythrocytes formed during the time of exposure to hypoxia at high altitude are destroyed preferentially upon return to normoxia. This was mainly indicated by a decreased number of reticulocytes, which paralleled the decrease in erythropoietin plasma levels. Because of this apparent specificity for young erythrocytes, i.e., the neocytes, this process has been termed “neocytolysis”. Unfortunately, experimental evidence from studies on human mountaineers is very weak and direct experimental proof is lacking. However, there seems to be some evidence for neocytolysis from mice exposed to hypoxia.

1.2. Aims: To show whether neocytolysis actually occurs upon descent after a prolonged stay at high altitude.

2. Methods: Twelve young adult male human subjects spent 3 weeks in the Jungfraujoch Research Station (JRS). Total hemoglobin mass (tHb) was measured with CO-rebreathing. Age-cohort labeling of erythrocytes was achieved by ingestion of 2 grams ^{13}C2- (pre-altitude-tests) and ^{15}N-labelled glycine in the middle of the stay at high altitude. ^{13}C and ^{15}N are naturally occurring, non-radioactive, stable isotopes. Glycine, and thus the isotopes, is incorporated into heme in hemoglobin-synthetizing erythroid precursor cells in the bone marrow. Blood was sampled following a time-course over approximately 140 days both, in the normoxic pre-altitude test and after ingestion at high altitude (thus, the entire study lasted for approximately 10 months). From these sequential blood samples, heme was isolated from the erythrocytes and ^{13}C/^{12}C- and ^{15}N/^{14}N ratios were measured by mass spectrometry (^{12}C and ^{14}N are the most common forms of C and N) for the quantification of the label and to follow the time course of erythrocyte removal and the life-span. Additional measurements included parameters of iron metabolism, of erythrocyte senescence, markers of hemolysis and erythrocyte clearance, erythrocyte redox state, membrane loss, ion balance and cell buoyant density, deformability, intracellular Ca, and erythroid stem cell proliferation.

3. Results: Only selected results are shown.

3.1. Results on erythropoiesis: Results show stimulation of erythropoiesis during the 30-day stay at the JRS indicated by elevated tHb (Figure 1) and elevated levels of erythropoietin in plasma (Epo) and elevated reticulocyte counts (Figure 2). The figures also show that all parameters decreased upon descent, and that tHb had nearly reached pre-altitude values 10 days after descent.

Figure 3 shows that the pattern of changes of the age-cohort label was the same in the pre-altitude (^{13}C/^{12}C) and after labeling at the JRS (^{15}N/^{14}N). This indicates that erythrocytes produced at high altitude mature with the same pattern as at low altitude. Also, erythrocyte-life-spans were not different.

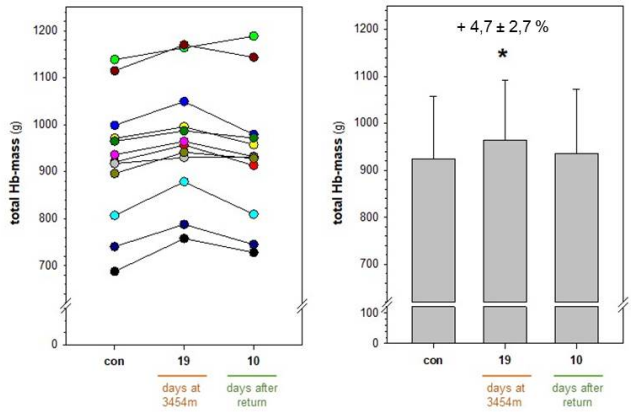

Figure 1. Change in total hemoglobin mass upon ascent and descent from the Jungfraujoch Research Station. Left: individual values; right: mean values ± SD.

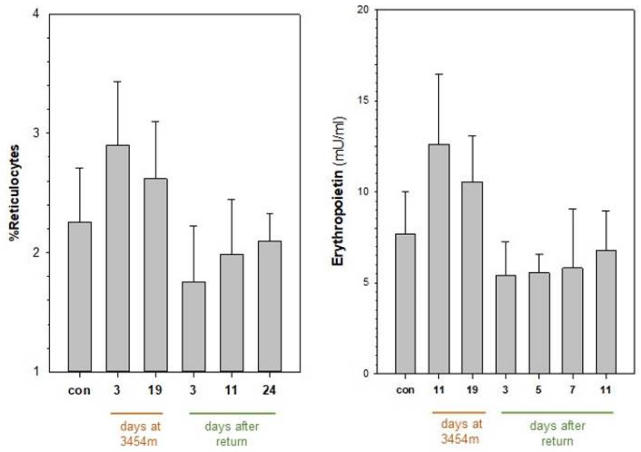

Figure 2. Change in reticulocyte counts (left) and plasma erythropoietin levels (right) upon ascent and descent from the Jungfraujoch Research Station. Mean values ± SD.

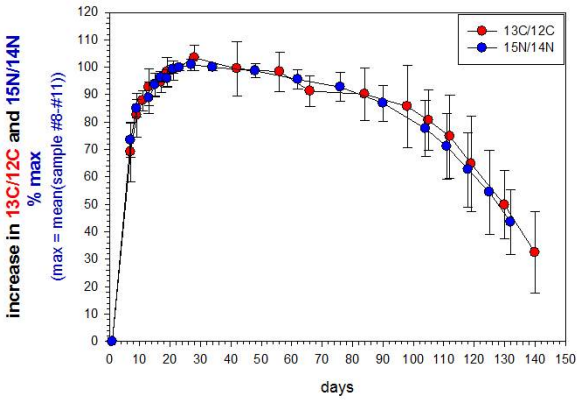

Figure 3. Change in erythrocyte age-cohort label in the pre- and post-altitude test. Pre-altitude erythroid precursors were labeled with ^{13}C2-glycine. On day 10 at the JRS, subjects ingested ^{15}N-glycine to label erythrocytes produced in hypoxia. Values of ^{13}C/^{12}C2- and ^{15}N/^{14}N-ratios were normalized to the highest intensity of the label (100%; ~ day 20 after ingestion). Mean values ± SD.

3.2. Results on erythrocyte age and senescence markers:

Altered erythrocyte membrane function and increased oxidative stress has been indicated to cause lysis of erythrocytes in mice after return from hypoxia to normoxia. Figure 4A indicates increased monobromobiname (MBBR) staining of thiols indicating elevated reductive stress upon ascent changing later to mild oxidative stress developing during the prolonged exposure to hypobaric hypoxia. Redox state was immediately restored to normal values upon descent. Figure 4B shows that phosphatidylserine exposure to the outer leaflet measured as annexin binding, which is elevated in senescent erythrocytes and serves as a clearance marker, actually decreased at the JRS and returned to normal levels after descent. Another marker of erythrocyte age is the ratio between the band 4.1a/4.1b erythrocyte-membrane protein-isoforms, where band 4.1a increases with erythrocyte age. Figure 5 shows a decrease in the band 4.1a/4.1b ratio at high altitude, which normalizes within a month after return to Heidelberg. Together, the results shown in figures 2, 3, 4 and 5 indicate a decrease in the mean age of the erythrocyte population at high altitude due to the stimulation of red cell production. Mean red cell age increased within a month upon descent, likely because of a reduced production rate in the first days after return from hypoxia to normoxia.

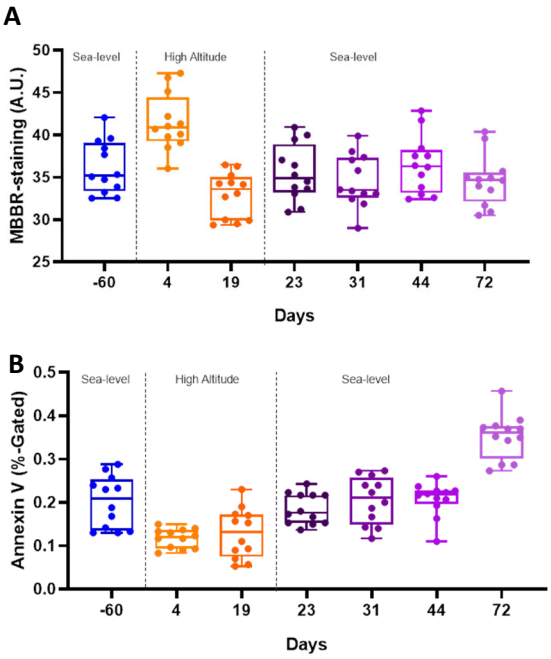

Figure 4. Senescence and clearance markers of erythrocytes in blood at low and high altitude. Panel A: Amount of reduced thiols (measure of redox state; both protein and non-protein ones, mono-bromobiname (MBBR) staining). Panel B: phosphatidylserine exposure to the erythrocyte membrane surface assessed by probing with annexin V.

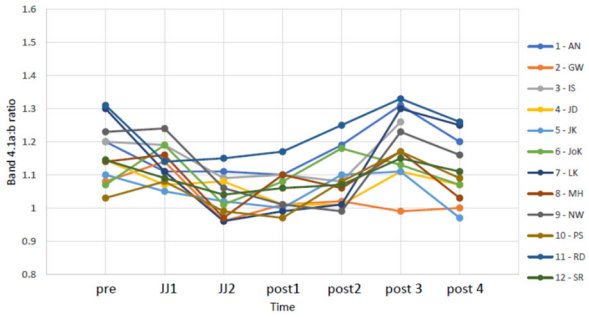

Figure 5. Individual values of changes in the erythrocyte membrane protein band 4.1a/4.1b ratios.

3.3. Results on stem cell maturation:

It is important to know whether erythroid progenitor cells differentiate and proliferate the same way in normoxia and hypoxia. Therefore, erythroid stem cells isolated from peripheral blood were matured *in vitro*. Figure 6 shows a representative result indicating that hypoxia during tissue culture does not affect the growth of erythroid progenitor cells *in vitro*.

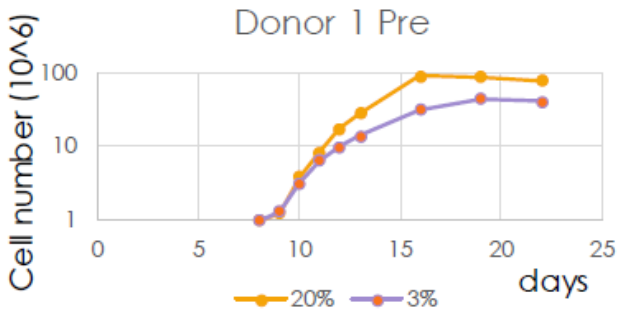

Figure 6. In-vitro differentiation and proliferation of erythroid precursor cells in normoxia (20% O_2) and hypoxia (3% O_2). Result from subject #1 from the pre-altitude test.

4. Discussion:

Our results, as expected, revealed an increase in total Hb-mass during the stay at high altitude, and a rapid decrease after return to low altitude. However, the pattern of changes of the age-cohort label of erythrocytes that had been produced at high altitude was not different from that of low altitude erythrocytes. This indicates that there was no selective destruction of erythrocytes produced at high altitude. Neocytolysis does not occur. Therefore, the reduction in total Hb-mass is likely, and most intuitively, a consequence of reduced erythrocyte production rates upon descent. This notion is supported by the decreased number of reticulocytes in the first days after descent. The decreased level of age and senescence marker protein 4.1R s in erythrocytes at high altitude persists after descent and indicates that young erythrocytes are still in circulation, and that they had not been destroyed as the neocytolysis-hypothesis would have predicted. Together with mathematical modelling (not shown here but in the manuscript) the results indicate that the low number of reticulocytes is solely due to a decrease in the erythropoietic rat, and the slow return of these parameters within the subsequent ~2-to-3 weeks to pre-altitude values indicates slow normalization of erythropoiesis. Therefore, a process like "neocytolysis" could not be detected and therefore most likely does not contribute to the decrease in tHb after descent from a stay at high altitude.

Collaborating partners / networks

Prof. Dr. Giampaolo Minetti, Department of Biology and Biotechnology, University of Pavia, Pavia, Italy
Prof. Dr. Silvia Rudloff, University of Gießen, Nutritional Sciences, Gießen, Germany
Dr. Emile van den Akker, Sanquin, Amsterdam, The Netherlands
Prof. Dr.Carsten Lundby, Inland Norway University of Applied Sciences, Lillehammer, Norway

Scientific publications and public outreach 2021

Refereed journal articles and their internet access

Kaestner, L., M. Klein, A.Y. Bogdanova, G. Minetti, S. Rudloff, C. Lundby, A. Mahkro, E. Seiler, A. van Cromvoirt, S. Fenk, G. Simionato, L. Hertz, S. Recktenwald, L. Schafer, T. Haider, S. Fried, C. Borsch, H.H. Marti, A. Sander, H. Mairbäurl, Neocytolisis: a space born concept of erythrocyte-removal falsified in the Swiss Alps, Flugmedizin Tropenmedizin, Reisemedizin, 25, 5, 232-236, doi: 10.1055/a-1524-7894, 2021.

Klein, M., L. Kaestner, A.Y. Bogdanova, G. Minetti, S. Rudloff, C. Lundby, A. Makhro, E. Seiler, A. van Cromvoirt, S. Fenk, G. Simionato, L. Hertz, S. Recktnwald, L. Schaefer, T. Haider, S. Fried, C. Borsch, H.H. Marti, A. Sander, H. Mairbäurl, Absence of neocytolysis in humans returning from a 3-week high-altitude sojourn, Acta Physiologica, doi: 10.1111/alpha.13647, 2021. https://onlinelibrary.wiley.com/doi/10.1111/apha.13647

Address

Translational Pneumology
UniversityHospital Heidelberg
Im Neuenheimer Feld 156
69120 Heidelberg
Germany

Contact

Prof. Dr. Heimo Mairbäurl
Tel. +49 6221 5639 329
e-mail: heimo.mairbaeurl@med.uni-heidelberg.de