Poster #200

Oxygen Levels In Vitro Affect B Cell Function through HIF-1A Signaling

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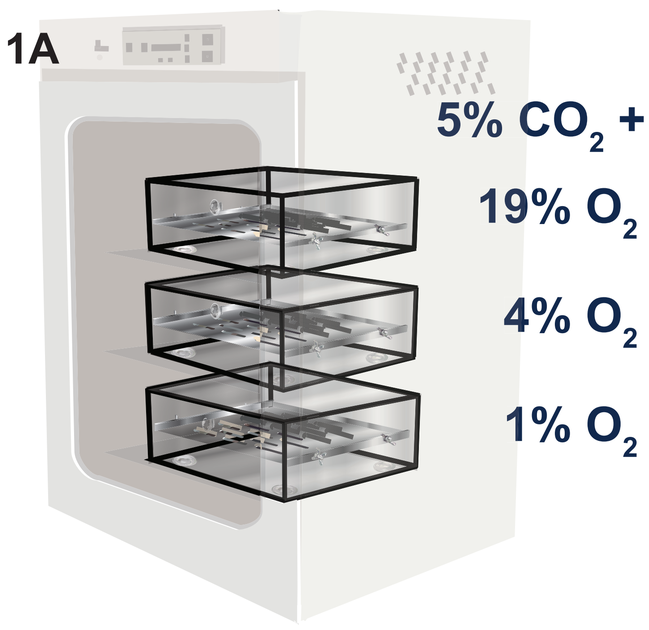
Abstract

Lymphocytes *in vivo* encounter highly variable oxygen levels as they migrate through different tissues, but all of those levels are far below room air. A standard CO_2 incubator does not contain 21% O_2, the oxygen level outdoors at sea level. High CO_2 and humidity drive incubator O_2 down (16-19%) and levels vary in response to door opening events. Oxygen *in vivo* is entirely dependent upon local physiology and cellular O_2 consumption rates (tissues 0-7%, blood 5-10%). The transcription factor hypoxia-induced factor 1 alpha (HIF-1a), a therapeutic target in multiple myeloma, is regulated at the protein level in response to changes in O_2 levels. Genes driving metabolism as well as proliferation, differentiation and migration, are downstream of HIF-1a. Here we compare the responses of B cells to culture at physiologic O_2 levels to those at room air incubator levels. We used C-chamber sub-chambers and Pro-Ox gas controllers to stably control the gas levels inside a standard incubator. We found that HIF-1a protein levels stabilized at physioxic 1% and 4%, but not at 19% O_2. B cell migration in response to the CXCR4 ligand, CXCL12, was significantly decreased in physioxia. Stable transfectants expressing a HIF-1a shRNA had reduced HIF-1a expression, even at low O_2. We found a correllating increase in CXCR4-mediated B cell migration as compared to a lentiviral control. This provides evidence that links *in vitro* O_2 levels, HIF-1a activity and B cell function, and suggests that physiologically relevant oxygen *in vitro* is critical.

Background

- **Oxygen levels in standard CO_2 incubators are highly variable and are not at room air nor physiologically relevant levels[1]**
- **Hypoxia-induced factor 1 alpha (HIF-1a) is regulated at the protein level and has an extraordinarily short half-life (~ 5 min) when cells are exposed to superphysiologic oxygen levels[2]**
- **Downstream of HIF-1a are genes critical to proliferation, differentiation, and migration as well as cell metabolism[3]**

Experimental Design



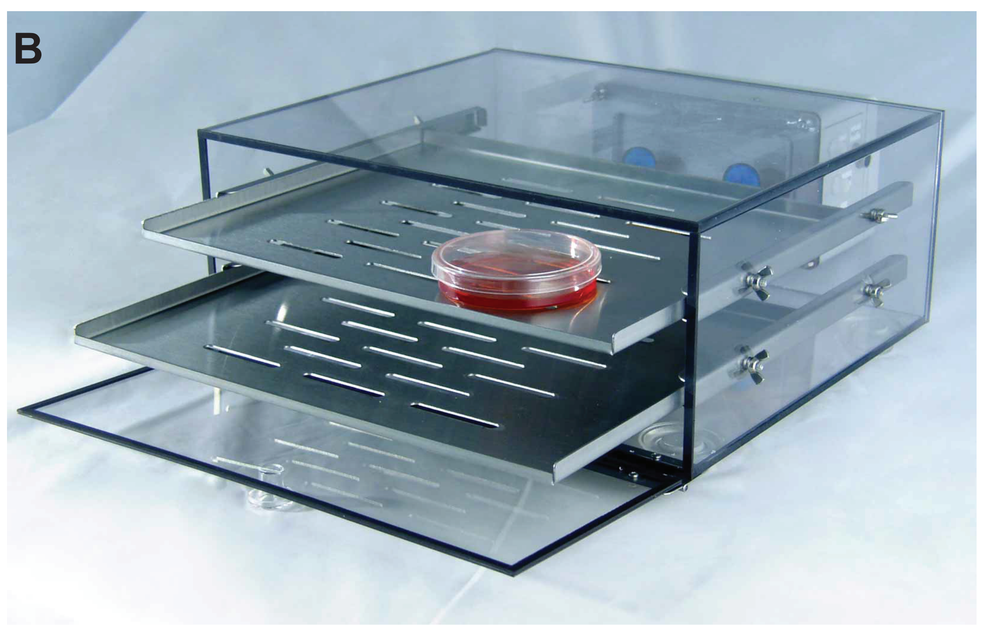
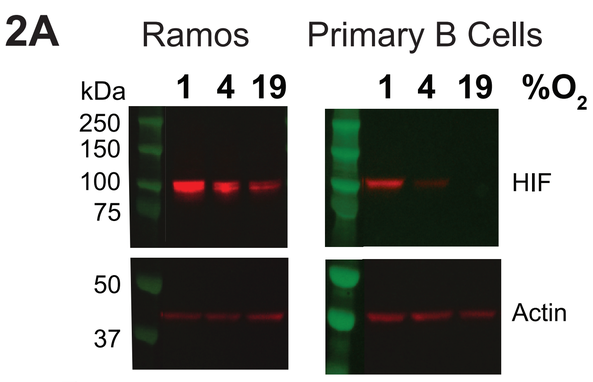
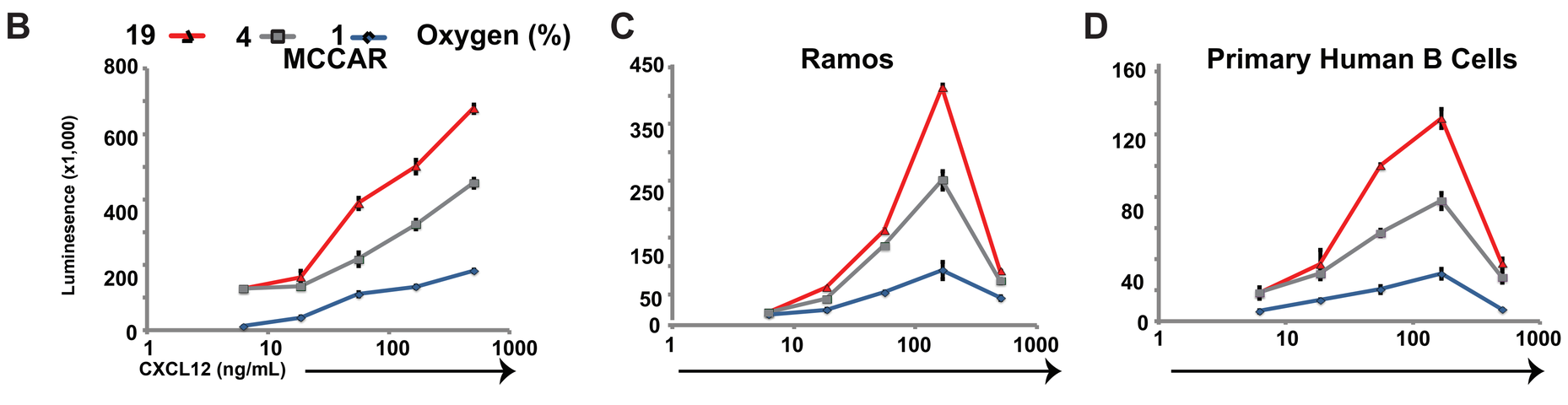
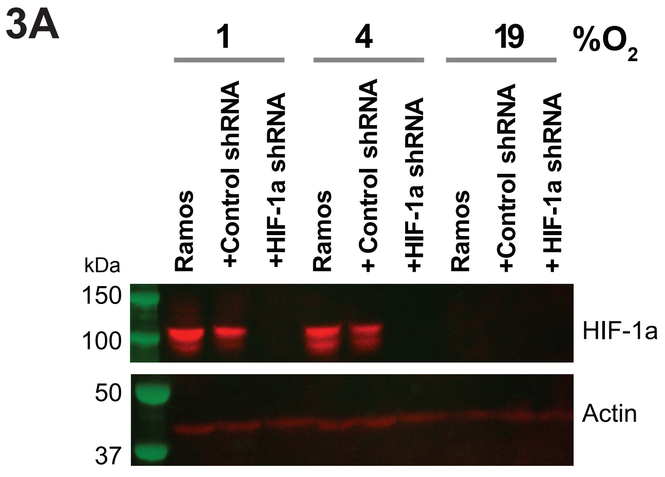


Figure 1. Experimental Design and Details
(A) Schematic. Primary human B cells or human lymphoma cell lines (stable HIF-1a transfectants selected with puromycin, or untransfected or lentivirus vector control) were incubated for 24 hrs at 1%, 4%, or 19% O_2 (incubator) levels plus 5% CO_2 using C-chamber incubator subchambers (B) and Pro-Ox gas controllers. Before use, all cell media (RPMI-1640+ 10%FBC (Gibco, USA)) were pre-equilbrated to appropriate oxygen levels. One gene downstream of HIF-1a in B lymphocytes is CXCR4. Cells were assayed for migratory response to a CXCR4 ligand, CXCL-12 (R&D Systems) for 1 hr using transwell plates with CellTiter-Glo (Promega) for detection. Nuclear extracts of cell lysates were subjected to western blot analysis for activated HIF-1a protein levels (mu-anti-HIF-1a (R&D Systems) HRP-gt-anti-mu (BD). Primary B cells were isolated from fresh human blood by positive selection (Miltenyi).

Results







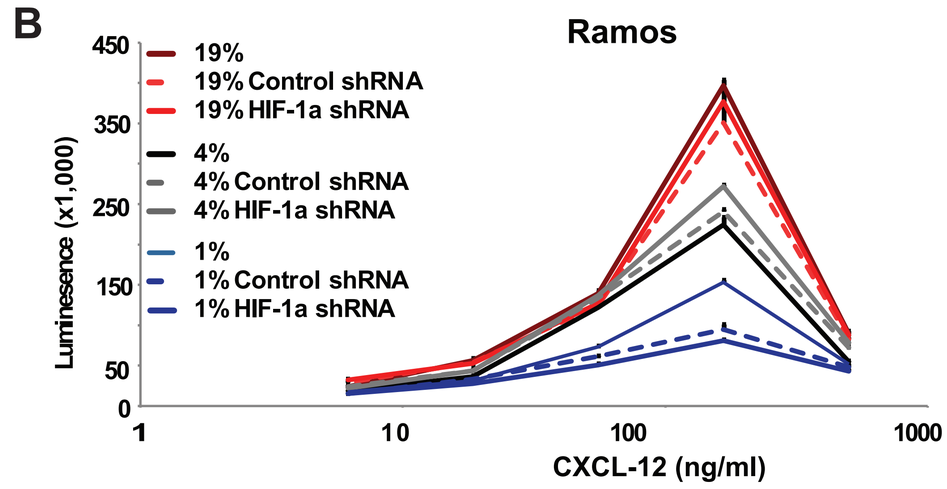


Figure 2. In Vitro Oxygen Levels Modulate HIF-1a and B Cell Migration. (A) As expected, HIF-1a levels were stabilized at 1 and 4% O_2 but reduced at 19% O_2. MC-CAR (B) and Ramos (C) cell lines, or primary B cells (D) were incubated at 1%, 4%, or 19% O_2. B cells migrated less in lower O_2, consistent with B cells migrating to and staying in low-oxygen bone marrow environments. Cell viability was unaffected.

Figure 3. Oxygen-Dependent B Cell Migration is Mediated HIF-1a (A) HIF1a-shRNA, but not control shRNA, reduced HIF-1a protein levels in Ramos even at low O_2. (B) Incubated at 1%, or 4% O_2, more Ramos transfected with HIF-1shRNA migrated in response to the CXCR4 ligand than control cells, indicating that HIF-1a is responsible for at least part of oxygen-dependent modification of cell migration.

Conclusions

Room air incubator oxygen modifies B cell function *in vitro* via HIF-1a.
Physiologically relevant oxygen levels *in vitro* are necessary for physiologically relevant results.

References

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[2] O. Frolova, I. Samudio, J.M. Benito, R. Jacamo, S.M. Kornblau, A. Markovic, W. Schober, H. Lu, Y.H. Qiu, D. Buglio, T. McQueen, S. Pierce, E. Shpall, S. Konoplev, D. Thomas, H. Kantarjian, R. Lock, M. Andreeff, M. Konopleva, Regulation of HIF-1alpha signaling and chemoresistance in acute lymphocytic leukemia under hypoxic conditions of the bone marrow microenvironment, Cancer biology & therapy, 13 (2012) 858-870.
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