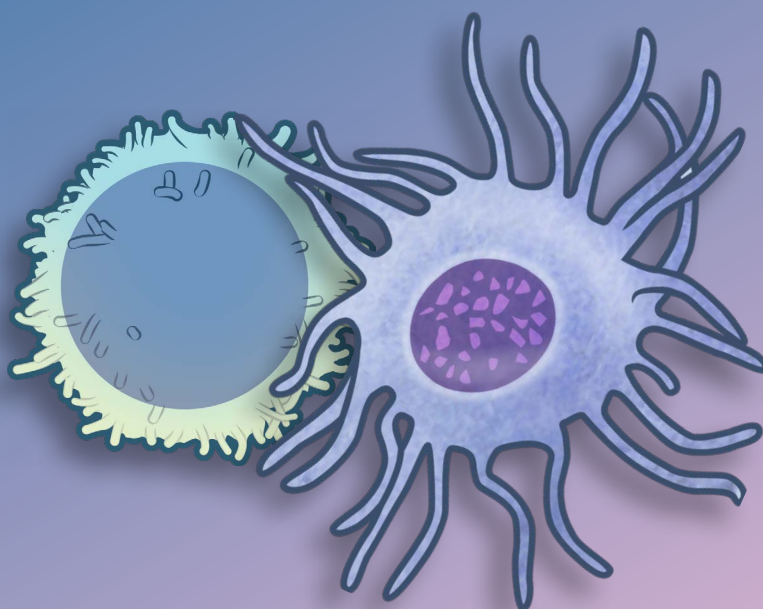




Technical Note

Immune Cells in Their Element: The Critical Role of Physiological Oxygen



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Introduction

In vitro studies using immune cells have traditionally relied on standard normoxic tissue culture conditions, which typically expose cells to atmospheric oxygen levels (~21% O₂). However, physiological oxygen concentrations within tissues — also known as physioxia — are significantly lower, ranging between 1–11% O₂ depending on the tissue and microenvironment. For immune cells, which operate in dynamic environments including lymphoid organs, peripheral tissues, and inflamed or tumor sites, the discrepancy between *in vitro* and *in vivo* oxygen levels can have profound implications. Accumulating evidence suggests that physiological oxygen levels are critical determinants of immune cell metabolism, differentiation, activation, and function. Hence, incorporating physioxic culture conditions into immunological research is not just a refinement, but a necessity for accurate biological modeling.

Oxygen Gradients in the Immune System

Immune cells operate across a wide range of oxygen tensions. For example, lymphoid organs such as the spleen and thymus have oxygen levels of around 1–5%, while inflamed or infected tissues may experience hypoxic conditions (<1% O₂). Tumor microenvironments are particularly hypoxic, which profoundly influences the behavior of tumor-infiltrating lymphocytes (TILs), macrophages, and myeloid-derived suppressor cells (MDSCs). Even within lymph nodes, the cortex and medulla exhibit distinct oxygen gradients, affecting immune cell positioning and function. Therefore, the physiological context of immune cells includes both spatial and temporal variations in oxygen tension, highlighting the need to recapitulate such conditions *in vitro*.

Hypoxia-inducible factors (HIFs), primarily HIF-1 α and HIF-2 α , are the central mediators of cellular adaptation to low oxygen levels. These transcription factors regulate hundreds of genes involved in glycolysis, angiogenesis, and immune responses, thereby influencing immune cell activity under physiological oxygen conditions, while absent at normoxic conditions.

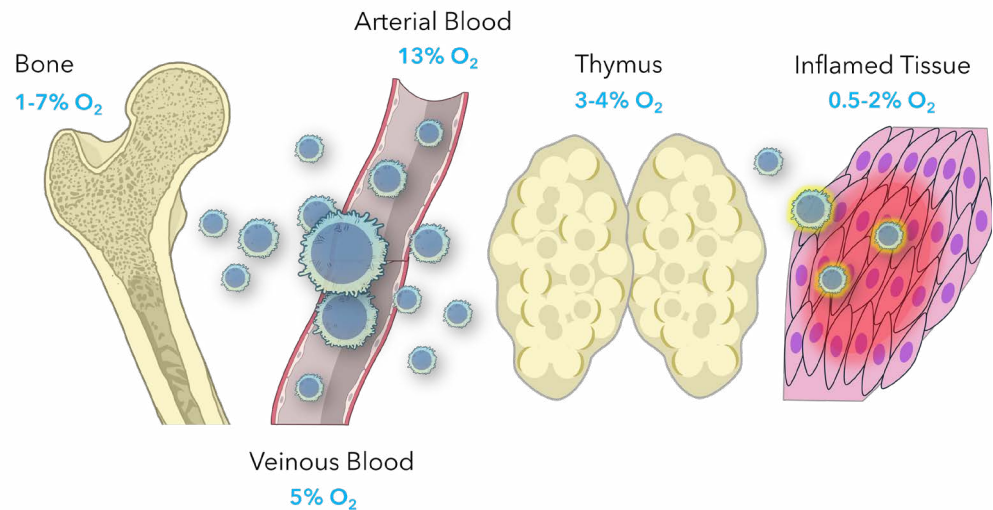


Fig. 1. An illustration of the variations in oxygen levels experienced by immune cells in different tissues.

Metabolic Regulation Under Physiological Oxygen

Immune cell activation is tightly linked to cellular metabolism. Naïve T cells, upon activation, undergo metabolic reprogramming from oxidative phosphorylation (OXPHOS) to aerobic glycolysis (Warburg effect), enabling rapid proliferation and effector function. B cells, macrophages, and dendritic cells also exhibit unique metabolic shifts upon activation. Oxygen availability regulates mitochondrial function, reactive oxygen species (ROS) production, and the stabilization of hypoxia-inducible factors (HIFs), particularly HIF-1 α and HIF-2 α .

Under physioxenic conditions, HIF-1 α is partially stabilized even in the absence of classical hypoxia ($<1\% \text{O}_2$), modulating gene expression involved in glycolysis, angiogenesis, and survival. For example, HIF-1 α drives the expression of glycolytic enzymes and inflammatory cytokines like IL-1 β and TNF- α in macrophages. In T cells, HIF signaling influences differentiation toward pro-inflammatory Th17 cells versus regulatory T cells (Tregs). Therefore, culturing immune cells under physioxenic conditions preserves physiologically relevant metabolic cues and signaling pathways.

Impact on T Cell Biology

T cells are particularly sensitive to oxygen tension during development, activation, and differentiation. Thymocytes in the thymus develop under low oxygen conditions (~1–5%), which influence T cell receptor (TCR) rearrangement and selection. Studies have shown that physioxenic culture of developing T cells more accurately reflects *in vivo* maturation patterns.

Upon activation, CD4⁺ and CD8⁺ T cells exhibit differential responses to oxygen levels. For instance, T cells cultured at 5% O₂ demonstrate enhanced proliferation and effector function compared to those at 21% O₂, partly due to more physiologic ROS levels and mitochondrial fitness. Furthermore, oxygen tension modulates the expression of checkpoint molecules like PD-1 and CTLA-4, with implications for immune exhaustion and immunotherapy.

T cell differentiation is also oxygen-sensitive. HIF-1 α promotes differentiation toward Th17 cells while repressing FoxP3⁺ Treg development. Thus, oxygen availability is a key factor in the balance of immune activation versus tolerance — a critical consideration in autoimmunity and cancer immunology.

Physioxenia and B Cell Development

B cell development occurs primarily in the bone marrow, a physiologically hypoxic environment with oxygen tensions ranging from 1–6%. This low-oxygen niche is essential for early B lymphopoiesis, including the transition from pro-B to pre-B and immature B cells. Hypoxia-inducible factors (particularly HIF-1 α) are key regulators of gene expression during B cell development, influencing survival, proliferation, and immunoglobulin gene rearrangement. Studies have shown that B cells cultured under physioxenic conditions exhibit improved viability and more faithful recapitulation of *in vivo* maturation stages. Moreover, during immune activation in secondary lymphoid organs, oxygen gradients can impact germinal center reactions, class-switch recombination, and plasma cell differentiation. For example, low oxygen enhances expression of activation-induced cytidine deaminase (AID), a critical enzyme for somatic hypermutation and class switching. Overall, physioxenia plays a fundamental role in shaping both early B cell development and antigen-driven differentiation, and its inclusion in B cell culture protocols enhances the physiological relevance of experimental outcomes.

Macrophages and Myeloid Cells

Macrophages exhibit remarkable plasticity, adopting M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes depending on environmental cues. Oxygen levels are a strong determinant of this polarization. Hypoxia or physioxia induces HIF-1 α -dependent expression of pro-inflammatory cytokines, nitric oxide synthase (iNOS), and glycolytic enzymes, promoting an M1 phenotype. Conversely, M2 polarization under normoxic conditions is associated with enhanced fatty acid oxidation and OXPHOS.

Importantly, the use of atmospheric O₂ in standard macrophage cultures may bias cells toward M2-like behavior and underestimate inflammatory potential. Myeloid-derived suppressor cells (MDSCs), which expand under hypoxic tumor conditions, also exhibit enhanced suppressive activity under low oxygen. Physiologically relevant O₂ levels are therefore essential to modeling myeloid cell behavior in tumor and infection settings.

Dendritic Cells and Antigen Presentation

Dendritic cells (DCs), as professional antigen-presenting cells, are responsible for priming naïve T cells and initiating adaptive immune responses. Oxygen tension affects DC maturation, antigen uptake, and cytokine production. For instance, hypoxia enhances DC migration via upregulation of CCR7 and supports IL-12 production, which promotes Th1 differentiation.

Culturing DCs under physioxic conditions improves their capacity for antigen processing and presentation, likely due to optimal balance between endosomal acidification and ROS generation. Additionally, the expression of co-stimulatory molecules like CD80/CD86 is more representative of *in vivo* activated DCs when cells are cultured at physiological oxygen levels.

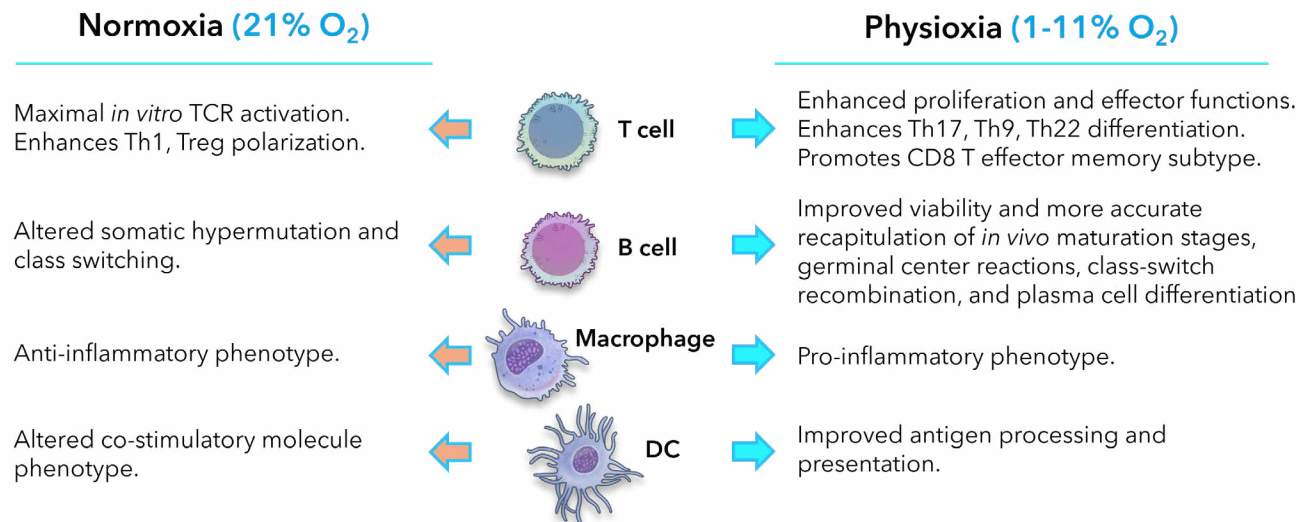


Fig. 2. Differences observed for T cells, B cells, macrophages, and dendritic cells, under normoxia versus physioxia.

Modeling the Tumor Microenvironment

In cancer immunology, the oxygen gradient within tumors poses a major barrier to immune infiltration and function. Hypoxia impairs T cell cytotoxicity, promotes regulatory cell accumulation, and fosters immune escape. Thus, replicating these conditions *in vitro* is crucial for evaluating immunotherapeutics such as checkpoint inhibitors and CAR-T cells.

Culture systems that incorporate physioxia or dynamic oxygen modulation allow for more accurate modeling of tumor–immune interactions. Moreover, they enable the study of hypoxia-induced resistance mechanisms and the development of oxygen-sensitizing strategies to improve immunotherapy outcomes.

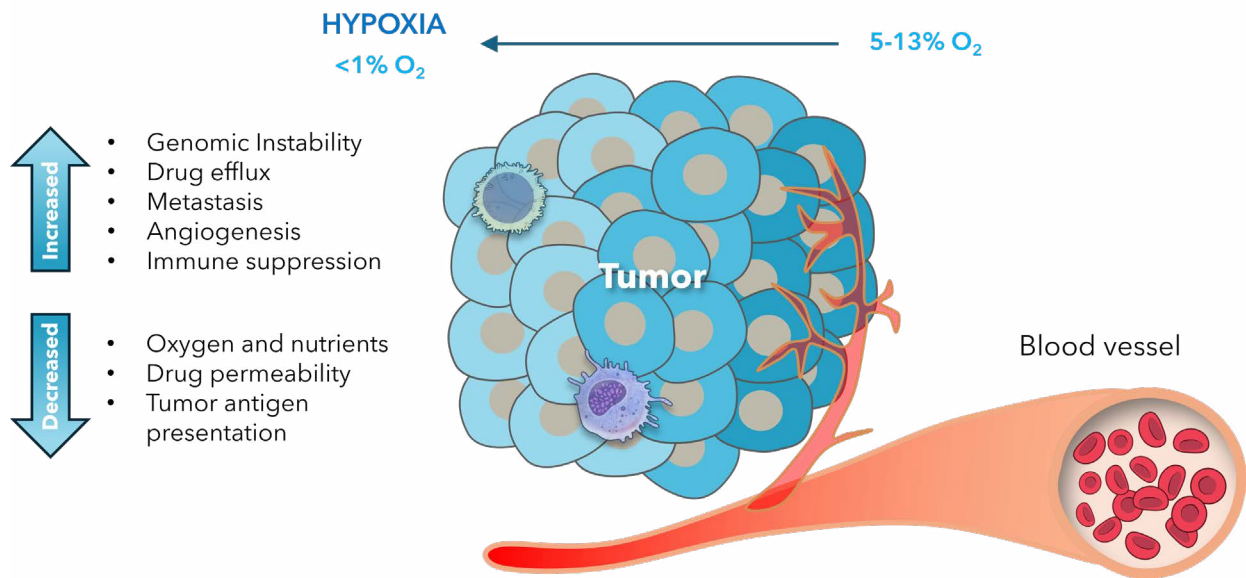


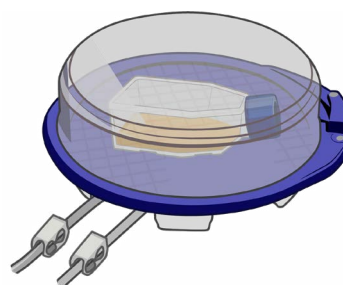
Fig. 3. Characteristics of the hypoxic tumor microenvironment.

Technical Considerations for Physioxic Culture

Implementing physioxic conditions in cell culture requires specialized incubators or hypoxia chambers with precise O₂ control. Media formulation, including buffering capacity and glucose levels, must be optimized to maintain pH and nutrient availability under altered oxygen tensions. Additionally, oxygen diffusion and consumption rates vary by cell type and density, necessitating careful experimental calibration.

Real-time monitoring of dissolved oxygen and inclusion of appropriate controls (e.g., normoxia and hypoxia) enhance reproducibility. Importantly, prolonged culture under non-physiological O₂ may cause irreversible cellular adaptations, underlining the need to adopt physioxia early in experimental design.

Learn more about how the [Modular Incubator Chamber](#) addresses physioxic and hypoxic cell culture requirements.



Conclusion

Physiological oxygen is a fundamental factor shaping immune cell behavior and function, which should not be overlooked. Oxygen tension profoundly influences immune cell metabolism, signaling, activation, and effector functions. Standard atmospheric oxygen conditions often fail to replicate the dynamic environments in which immune responses occur *in vivo*. Incorporating physioxic culture systems into immunological research enhances the physiological relevance of *in vitro* models and provides more accurate insights into immune regulation, disease pathology, and therapeutic efficacy. As our understanding of oxygen's role in immunity continues to evolve, so too must our experimental paradigms.

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