REVIEW ARTICLE

# Physiological, *ex vivo* cell oxygenation is necessary for a true insight into cytokine biology

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Eukaryotic physiology is founded upon aerobic metabolism. This results in the synthesis of highly energetic phosphates, that is highly dependent upon the presence of oxygen ($O_2$). However, the $O_2$ concentration must be adapted to the particular metabolic type of cell, which is why one of the major functions of the respiratory system and circulation is to provide an appropriate $O_2$ concentration for each cell type. In the human organism, the $O_2$ concentration varies significantly between the tissues: in the circulation and in lung parenchyma [1-4], as well as in well-irrigated, parenchymal organs (liver, kidneys, heart) [5-9], it is between 4 and 14%. In other tissues, relatively less well-irrigated, the $O_2$ concentration is even lower: in the brain, it varies from 0.5 to 7% [10-12], in the eye (retina, corpus vitreous), from 1 to 5% [13] (reviewed in [14]), and in bone marrow, from 0 to 4% [15, 16].

For most cell types, the optimal $O_2$ concentration is between 3 and 6%, *i.e*. the concentration existing in the atmosphere approximately 1 billion years ago [17, 18]. Some cell types should even be protected from $O_2$, and need concentrations lower than 2% possibly approaching zero, corresponding to atmospheric concentrations between 2 and 3 billion years ago. This early step in evolution [21, 22] is reflected by the metabolic properties and ontogenesis of primitive stem cells [19, 20]. Indeed, $O_2$ favours synthesis of reactive oxygen species (ROS) that might have a regulatory role, but which could also be harmful to the cells. Every cell type exhibits a system of antioxidant defence.

In fact, in course of evolution, cell metabolism has become adapted to moderate oxygenation *i.e*. oxygen concentrations that can be found in the tissues [21, 22]. This adaptation has involved both the modulation of the molecular mechanisms existing in living organisms before appearance of oxygen in the atmosphere, and adoption and development of new mechanisms such as cellular respiration (reviewed in [24]) during the course of oxygenation of the atmosphere (reviewed in [23]). For example, the stabilisation of HIF-1α transcripts, which originally initiated synthesis of molecules acting in anaerobic metabolism, shifted towards their oxygen-dependent degradation.

Thus, eukaryotic cell metabolism must find the balance between the need for oxygen to provide energy in the form of energetic phosphates, and the fight against excess ROS. This system however, has its limits, and to ensure the well-being of cells, oxygen concentrations should not go beyond them [25]. In this respect, it is clear that the atmospheric $O_2$ concentration is too high for the cells of most tissues.

Nevertheless, almost all present knowledge related to the action of cytokines on cells, and to the consequent cellular response, is based upon experiments performed at 20-21% $O_2$ *i.e*. highly hyperoxic conditions. Apart from the technical problems related to controlling $O_2$ concentrations, it is likely that two paradigms were at the origin of this negligence in the matter of *ex-vivo* culture oxygenation: *(i)* erythropietin (Epo)-dependent regulation of red blood cell (RBC) production and *(ii)* vascular endothelial growth factor (VEGF) and its role in neovascularisation. These two paradigms led to a mental "shortcut", referring only to the "hypoxic condition" within the biology of these two factors. From the late seventies until today however, a number of data have been collected, clearly demonstrating that the cellular response to cytokines depends upon the actual $O_2$ concentration. For example, in the same culture medium and in the presence of the same soluble factors, haematopoietic cells have shown a completely different response at low $O_2$ concentration compared to that at 20-21% [26-30]. The real nature of stem cells *i.e*. their maintenance in G0 phase, self-renewal, commitment, etc., which are, of course, regulated by certain cytokines and growth factors, should be re-evaluated at the appropriately low $O_2$ concentrations that characterize the stem cell niche [31-33]. Thus, here again, the response of stem cells to cytokine stimulation is completely different at 0.1, 1, 3-5, than at 20% $O_2$, implying a physiological, regulatory role for oxygen concentration in early haematopoiesis [34-37]. For example, we recently demonstrated a positive effect of IL-6 on stem cell maintenance. However, this was revealed only at 1% and not at 20-21% $O_2$ [38]. The response of committed progenitors to cytokine stimulation involves all haematopoietic lineages [39-41]. It was shown for example that $O_2$ tension alters the effect of cytokines on mega-

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karyocyte, erythrocyte, and granulocyte lineage [39]. As a matter of fact, the findings at 3-5% $O_2$ should be taken as the physiologically normal condition, and 20-21% as a non-physiological, hyperoxic state. This point has been confirmed in the *ex vivo* model of erythropoiesis, where low $O_2$ concentrations seem to be a general physiological regulator, beyond the well-known, Epo-related downstream tuning [42, 43], (submitted). Recently, a series of papers studying different cell types have confirm that the actual $O_2$ concentration determines the cellular response to cytokines as well as the cytokine secretion pattern of the cells [44-58]. In line with these studies are some papers published in ECN, a recent one by Schutyser [59] and another by Krstic *et al*. (page 10 to 16 of this issue). The latter reveals that the extent of IL-17 action on CD34+ cells and haematopoietic progenitors, is $O_2$ concentration-dependent. Furthermore, the stimulating effect of IL-17 on BFU-E was markedly enhanced at 3% $O_2$ (assumed to be the physiological $O_2$ concentration in progenitors residing in bone marrow). This is yet one more reason to urge the revision of our knowledge of cellular responses to cytokines that has been acquired at non-physiological, atmospheric (20%), $O_2$ concentrations. Low oxygen, without doubt, better approximates the *in vivo* environment, *i.e*. "*in situ* normoxia".

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