

HYPEROXIA INHIBITS T CELL ACTIVATION IN MICE

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ABSTRACT

The immune response is blunted in mice and humans in spaceflight. The effects of hyperoxia in mice alter expression of some of the same immune response genes. If these two conditions are additive, there could be an increased risk of infection in long duration missions.

Background: Immunosuppression is seen in healthy astronauts who have flown in space; however little is known about the mechanisms that cause the reduced immunity in spaceflight. Here we examine the role of oxidative stress on mice exposed to periods of high O₂ levels mimicking pre-breathing protocols and extravehicular activity (EVA). To prevent decompression sickness, astronauts are exposed to elevated oxygen (hyperoxia) before and during EVA activities. Spaceflight missions may entail up to 24 hours of EVA per crewmember per week to perform construction and maintenance tasks. The effectiveness and success of these missions depends on designing EVA systems and protocols that maximize human performance and efficiency while minimizing health and safety risks for crewmembers. To our knowledge, no studies have been conducted on the immune system under 100% oxygen exposures to determine the potential for immune compromise due to prolonged and repeated EVAs.

Methods: Animals were exposed to hyperoxic or control conditions for 8 hours per day over a period of 3 days, initiated 4 hours into the dark cycle (12h dark/12h light), using animal environmental control cabinets and oxygen controller (Biospherix, Lacona, NY). Experimental mice were exposed to 98-100% oxygen as a model for pre-breathing and EVA conditions, while control mice were maintained in chambers supplied with compressed air. These are ground control studies where we use real-time RTPCR (qRTPCR) to measure gene expression of the early immune gene expression during bead activation of splenocytes of normoxic and hyperoxic mice. All procedures were reviewed and approved by the IACUC at Ames Research Center. After the last 8h of hyperoxic exposure, spleens were removed and the splenocytes were isolated and kept as individual biological samples. We have also examined transcription factors (JASPAR) and pathways of the immune system to help us understand the mechanism of regulation.

Results: Our recent mouse immunology experiment aboard STS-131 suggests that the early T cell immune response was inhibited in animals that have been exposed to spaceflight, even 24 hours after return to earth. Moreover, recent experiments in hyperoxic mice show that many of the same genes involved in early T cell activation were altered. Specifically, expression of IL-2R α , Cxcl2, TNF α , FGF2, LTA and BCL2 genes are dysregulated in mice exposed to hyperoxia.

Conclusions: If these hyperoxia-induced changes of gene expression in early T cell activation are additive to the changes seen in the microgravity of spaceflight, there could be an increased infection risk to EVA astronauts, which should be addressed prior to conducting a Mars or other long-term mission.

INTRODUCTION: Oxidative damage caused by hyperoxia is a long known risk to retinal and lung tissue, however, little is known of its impact on the immune system. In animals, prolonged hyperoxia causes histopathological changes similar to those seen in acute respiratory distress syndrome and models of ventilator-induced lung injury (1). It is assumed that hyperoxic toxicity is mediated by excess production of reactive oxygen species (ROS)(2). Oxidative damage caused by hyperoxic conditions preceding or during extra vehicular activities (EVA) may well have an impact on crew health during spaceflight.

ROS studies in immunology previously focused on their role in antimicrobial defense as part of the innate immune system (3). In this presentation, we report the relative impact of intermittent hyperoxia on C57BL/6J mice to mimic the hyperoxic conditions of pre-breath and EVA periods in astronauts in the adaptive immune system. We present preliminary evidence testing the hypothesis that EVA-like hyperoxia causes alterations in oxidative stress related gene expression, and immune regulation. We report changes in gene expression of splenocytes are in some ways related to changes in T cell activation that we have previously documented in space flight on STS-131.

RESULTS: Response of gene expression in the adaptive immune system in hyperoxic animals were studied. Preliminary data suggests a separate mechanism for reduced T cell activation in hyperoxia as compared to microgravity (μ g).

Gene	NA	Act 1G	Act µg	Act HyOx
IL-2	-	+++++	++++	ns
IL-2R α	-	+++	++	++
IFN γ	-	+++	++	ns
TNF α	-	+++	++	+
BCL	-	+	+	-
SOD	-	+	-	-

Table I: Significant Relative expression of key genes in immune response in normal g, µg or hyperoxia

Splenocytes were taken from animals that were ground controls, in spaceflight, or exposed to hyperoxia. Splenocytes were activated with CD3/CD28 coated beads. NA: not activated, Act1G ground animals; Act µg: spaceflown animals; Act HyOx (ground) animals exposed to hyperoxia for intermittent 12 hours intervals. For significantly changed genes as compared to ground controls: +++++ =>1000 fold increase in gene expression; ++++ => 100 fold gene expression; +++ =>20 fold increase; ++ => 5 fold increase; + =>2 fold increase; - = NA control levels. Ns=non significant change

As seen in Table I, gene expression of key immune genes IL-2 and IFN γ were not affected by hyperoxia, suggesting that unlike µg, the hyperoxic effect is post TCR activation step making it a different mechanism of regulation.

DISCUSSION: Long duration spaceflight missions may entail up to 24 hours of EVA per crewmember per week to perform construction and maintenance tasks. In addition, there are some proposals that include having two separate compartments one with up to 40% O₂ (under reduced psi) to reduce pre-breathing periods before EVA. The effectiveness and success of these missions depends on designing EVA systems and protocols that maximize human performance and efficiency while minimizing health and safety risks for crewmembers.

To our knowledge, there have been no studies evaluating the effects of increased O₂ levels on the immune system as in prolonged and repeated EVAs. This study using 100% O₂ is the first step to understand the effect of altered gas mixes on immune function. Previous reports from Shuttle flights demonstrated a change in the immune system components (4-6). Spaceflight causes changes in innate immune function as well as adaptive immune function (7, 8). If reduced T cell activation due to microgravity and increased oxygen have different mechanisms of action, it is possible that exposure to hyperoxia during EVA in microgravity may compound the total impact on immune function of astronauts.

T cell activation causes a sequential expression of key immune genes (7), which leads to a fully functional adaptive immune response. Activation has three steps necessary for full T cell activation, the first two cause synthesis of IL-2 which binds to the constitutive low

affinity IL-2R (β,γ subunits) thereby up-regulating the third receptor, IL-2R α , which completes the high affinity IL-2R receptor. It is likely that the effects of spaceflight and hyperoxia are additive and therefore the combined effects of hyperoxia and microgravity should be more fully studied to mitigate the risk to crews during long term spaceflight. These findings also may help in our understanding of the growing problem of significantly increased hospital infections of patients treated with hyperoxic mechanical ventilation.

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