HUMAN SPACE BIOLOGY AT SCK•CEN: FROM *IN VITRO* CELL EXPERIMENTS TO THE FOLLOW-UP OF ASTRONAUTS

Background

Prolonged exposure to space radiation and extended microgravity has revealed profound physiological and clinical changes in astronauts.

The health problems thought to be related to the effects of ***microgravity*** include a decrease in the heart and the respiratory rates, a loss of body weight, changes in bone calcium, a redistribution of body fluids with a greater amount in the upper body, a decrease in muscle tissue, a weakening of the veins and arteries in the legs, as well as an underproduction of red blood cells leading to anaemia. At the cellular and molecular levels, microgravity is known to induce both a loss of T-cell activation and changes in gene expression patterns, as well as a three-dimensional growth of normal cells and tumour cells, an alteration of the mitochondrial organization, a modification of the production of extracellular matrix proteins and apoptosis in some types of cells.

The Earth's magnetic field protects us from harmful ***radiation***. On Earth, we are still exposed to small amounts of radiation when we go for medical x-rays, when we travel on transcontinental flights or just from radon in the air. However, astronauts are exposed to 50 to 100 times as much radiation - and that is just in a low Earth orbit. In deep space, astronauts can be exposed to even higher doses. It is well known that large amounts of radiation can cause severe health effects by altering DNA in our cells. The health effects from space radiation are therefore a critical safety concern for long-term space travel. Possible health risks include cancer, cataracts, acute radiation sickness, hereditary effects, and damage to the central nervous system.

Objectives

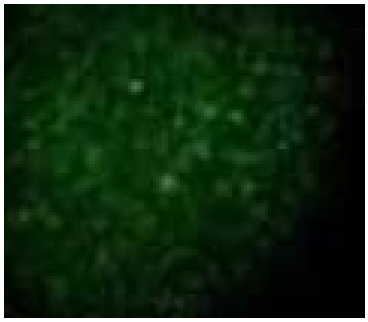
The aims of this research are 1) to ensure the immunological monitoring of a cohort of astronauts (having spent around 6 months aboard the International Space Station ISS) and 2) to investigate the effects of an *in vitro* exposure of endothelial cells and other types of cells to radiation and/or microgravity conditions.

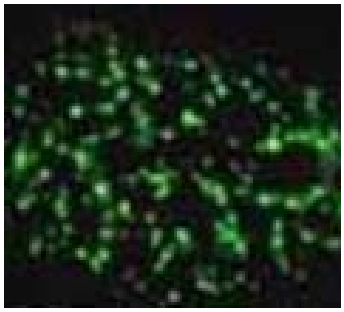
Aim 1): Within the IMMUNO project (coordinator: Dr. A. Chouker, Munich, D), supported by the European Space Agency (ESA), a comprehensive biochemical analysis supplemented by psychological tests to investigate immune system changes in ISS long-term crews is being conducted. By comparing this data with studies of patients who are isolated or confined to bed, we expect to gain insight into the role of the various factors which weaken the immune system, as well as into the mechanism of the immune defence. Such knowledge constitutes the foundation of the development of new preventative or therapeutic methods, which may then be used equally on astronauts or on critically ill patients in intensive care. The aim of the ESA IMMUNO project is to determine the changes in stress and immune responses, during and after a long stay aboard the ISS. This involves the sampling of saliva, blood and urine to check for hormones associated with stress response and for radiation-induced DNA damage in cells.

Aim 2): While one possibility is that the unique physical and psychological stresses of space flight might trigger immune-altering hormones, another possibility is that the weightlessness of space might be affecting the immune cells directly. The "rotating bioreactor" available at SCK•CEN (and represented in the second set of figures below) will help to define the possible effects of microgravity on cells without the complications of hormone effects. It is a rotating container full of fluid which allows cells to remain suspended for months at a time in continual free fall, just as they would be in Earth orbit. This system coupled to irradiation allows us to discriminate precisely between the effects of microgravity and irradiation on cells in the *in vitro* conditions.

Principal results

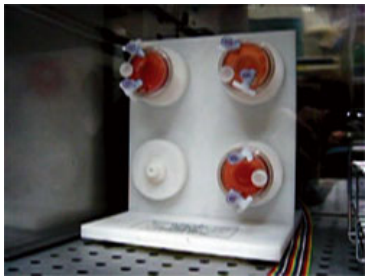
The IMMUNO project involves making several pre-flight, in-flight and post-flight measurements on astronauts. Blood collections are conducted to permit the analysis of blood and its various components. This project is carried out over multiple ISS increment astronauts, requiring at least six subjects in total. So far, three astronauts have participated in the study. Hormone concentrations are measured in the serum samples using the multiplex array assay technology available at SCK•CEN. Furthermore, the extent of DNA damage in the astronauts' lymphocytes is determined as shown below.

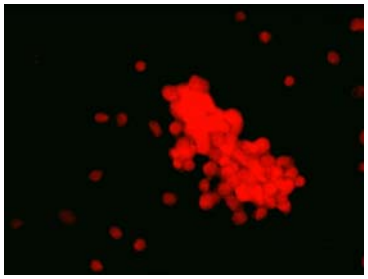


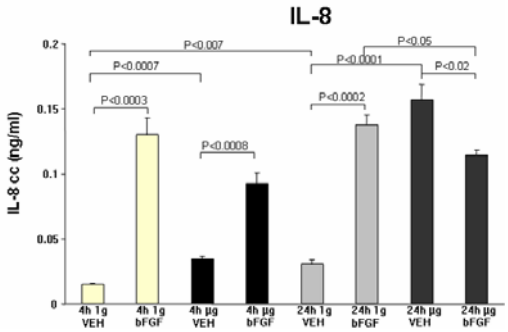


DNA damage in astronaut lymphocytes is revealed by γH2AX fluorescence. H2AX is phosphorylated by various proteins involved in the response to DNA damage (like ATM, ATR and DNA-PK). Its phosphorylated form (γH2AX) constitutes the marker of DNA double strand breaks (typical of radiation). In the left picture a nucleus of a control lymphocyte, in the right a nucleus from a lymphocyte 2 hours after X-irradiation with 10Gy.
The green dots present in the right figure reveal the extent of DNA damage.

Endothelial cells respond to changes in blood pressure, oxygen tension and blood flow by secreting substances with powerful effects on the tone of vascular smooth muscle. In response to adverse stimuli such as wounds, infections or tumour challenge, the endothelial cells are activated and change their functions. Furthermore, they contribute to the formation of new vessels (tissue regeneration). Microgravity applied on endothelial cells induces the formation of multicellular spheroids/ tubular structures (shown below) within a short time, alters the cytoskeleton, induces apoptosis (casp 3, TUNEL) and gene expression of Fas and FasL as well as changes in the secretion of various cytokines (ET1 vasoconstrictor and cell proliferation, Eotaxin, ICAM-1, cell adhesion, C-reactive protein, IL-8, IL-12, IL-15, VEGF) as shown by the recent multiplex array (Luminex) technology used at SCK•CEN.







This figure depicts on the left, the bioreactor used to induce microgravity conditions; in the middle, endothelial cells forming spheroids; on the right, one of the hormone (here interleukin-8) measurements performed with our multiplex array assay (Luminex) facilities.

Future developments

Either under the conditions of space flight/microgravity or selective radiation or microgravity challenges in human cell-systems, the characterization of new physiological or pathological processes induced in space will be possible. In that way, the determination of many pro- and anti-inflammatory cytokines, tissue hormones and other factors orchestrating the host defence, the cell- to cell- communication, cell repair or cell death altogether will allow a broad extent of knowledge of space-related health effects.

Main contact person

Sarah Baatout, sarah.baatout@sckcen.be

Main references

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