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Preliminary Results on Cosmic Radiation Effects in T Lymphocytes

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Abstract

Experiments carried out in space and in clinostate demonstrated the influence of microgravity on mitogenic activation of human T lymphocytes at molecular level. To discriminate between effects of microgravity and cosmic radiations, in this work we studied the effects of cosmic radiations on the genetic expression in human T cells boarded in a stratospheric balloon (BIRBA-1 mission, 22 hours of flight). The genetic expression was analyzed by the cDNA microarray, which allows the comparative and simultaneous estimate of hundreds of mRNAs. Activated cells react to the ionizing stress by activating genes involved in cell cycle check-point, oxidative stress response, heat shock proteins production or by repressing genes involved in antigen recognition.

Introduction

The effect of space conditions on human T cells activation has been extensively studied for the last twenty years. In space living organisms, including cells, are affected by two new environmental conditions: microgravity and cosmic radiations. Recently we demonstrated that the *in vitro* mitogenic activation of human T lymphocytes is remarkably depressed in microgravity (3): the process involves the binding of Con A to membrane glycoproteins followed by patching and capping (4), cell-cell interactions and the production of IL-1 and IL-2 (5). Since upon interaction between IL-2 and its receptor (IL-2-R) the full activation is triggered, recently we studied the genetic expression of IL-1, IL-2 and IL-2-R, early genes and IFN gamma in this process using a new three-dimensional clinostat (RPM) to simulate microgravity (6). The molecular understanding of these processes and the recognizing of related counter-measures could be remarkably important for the human activity continuation in the space.

Since ionizing radiations are of fundamental interest for space missions and International Space Station utilization, the aim of this work was to discriminate between the effects of microgravity and cosmic radiation in T cells. In order to evaluate the effects of cosmic radiations on the gene expression in human T lymphocytes we exposed these cells to high quote cosmic radiations during a stratospheric balloon trans-Mediterranean flight. The genetic expression was analyzed by the cDNA microarray hybridization technology, which allows the comparative and simultaneous estimation of hundreds of mRNAs (1).

Methods

T-lymphocytes from human peripheral blood (HCV, HIV 1/2, Abs Ag, treponema negative) were obtained by gradient centrifugation on Histopaque-1077 according to Boyum method (2) and purified by human T cell enrichment columns (R&D System) via high affinity negative selection. Erythrocytes were lysed with ACT (Ammonium Chloride solution Tris buffered) preparation. Cell viability was assessed by trypan blue dye exclusion test. T lymphocytes were resuspended at a concentration of 10^6 /ml in RPMI-1640 supplemented with 40 mM HEPES, 5mM sodium bicarbonate, 4 mM L-glutamine, gentamycin (50 μg/ml), 10% heat-inactivated fetal calf serum (GIBCO) and activated by addition of Con A (10 μg/ml), purified mouse anti-human monoclonal antibody CD 28 (4 μg /ml) and protein G (2 μg / ml) about 8 h before the balloon launch (BIRBA-1 mission, Trapani Milo, Italy, July 2000). Activated (A) and non activated (NA) cell cultures were sealed in 10 ml Falcon tubes and boarded in the balloon (Fig. 1).

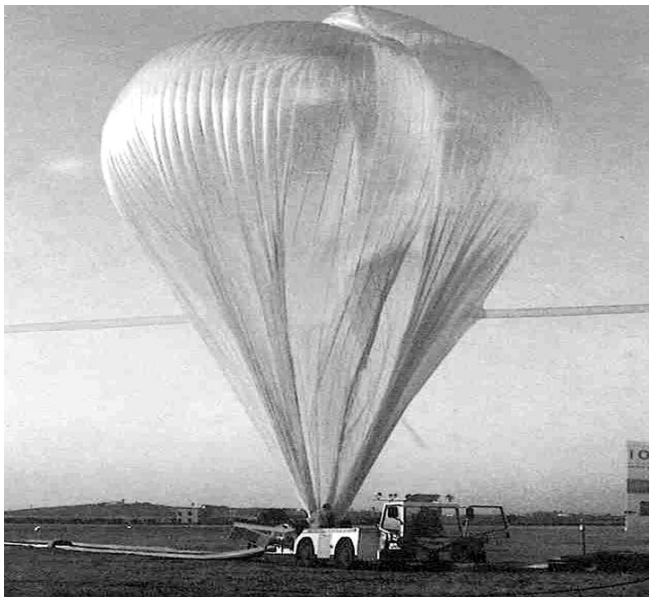

Fig. 1 - The stratospheric balloon "BIRBA 1"

Two cell samples (A+NA each) were set inside two special containers (Kayser®, Italy) designed to maintain them in

suitable and controlled conditions of temperature (37±1°C) and pressure (1±0.05 bar) during the flight. One of the two containers was partially shielded from cosmic radiations (fast neutrons component) to possibly detect eventual differential biological effects of radiation dose and composition. An accurate dosimeter system for gamma rays, thermal neutrons and fast neutron components was present on board (Table 1.). Thanks to the dosimeter data and to a simulation system (A. Zanini, INFN, Torino) a reliable estimation of the biological samples total exposure was made possible: it amounted to 8.439±20% mSv/h in the unshielded and to 4.789±20% mSv/h in the shielded container. It should be noted that we registered an increase over the programmed temperature for several hours during the flight in the unshielded container. After 22 hours of flight the balloon landed in Spain where the biological samples were recovered and transported to the local host laboratory. Lymphocytes were immediately processed for mRNA extraction with TRIZOL, 14 h after landing. A third sample of T-cells (A+NA) was used as control on the ground. Purified RNA was converted to ^{33}P-labeled cDNA and used to hybridize human gene filters microarrays. Control gene filters microarrays were hybridized with RNA extracted with samples kept at ground in the same conditions.

For the cDNA microarrays analysis we used the Research Genetics Human GeneFilters microarrays, single membranes (5cm x 7cm) containing approximately 4,000 genes, all of which are of known function. Probe preparation and filters hybridization were performed as recommended by the manufacturer. Hybridized filters were placed in cassettes containing storage phosphor screens (Packard). Various exposures were performed for each experiment. Exposed screens were scanned by the Packard Cyclone system at a resolution of 600 DPI. Digitized images so obtained were analyzed and compared using the Pathways^{TM} software developed by Research Genetics. The software calculates and subtracts the background and normalizes the data points intensities

Table 1 - Radiation values in pressurized boxes

Cosmic radiations	Unshielded box	Shielded box
γ Radiation	2.2 μSv/h	2.2 μSv/h
Ray X	0	0
Thermal neutrons (0.025 eV-100 eV)	0.02 μSv/h	0.01 μSv/h
Epithermal neutrons (100 eV-10 keV)	0.019 μSv/h	0.009 μSv/h
Fast neutrons (10keV-20MeV)	4.15 μSv/h	2.02 μSv/h
*Relative neutrons (up 20 MeV)	0.05 μSv/h	0.05 μSv/h
*Heavy ions	2 μSv/h	0.5 μSv/h
Total	8.439 μSv/h	4.789 μSv/h

*There are not instruments for the measure of heavy ions and the relative neutrons; relative quantity was deduct by literature and calculus

Results and discussion

Several genes were found to be induced in the activated T-lymphocytes (Table 1). In this case the inductions were more evident in the cells from the unshielded container: a possible explanation being in the higher resistance of T lymphocytes to heat, as compared with other cells (HUVEC) utilized from others researchers in the same mission. The most interesting aspect of the data is that a subset of the genes is induced in both kinds of cells (data not shown). Most of them were already known to be induced by ionizing radiation or to play a role in the cellular response to genotoxic stress (7). Altogether, activated T cells react to the ionizing stress by activating genes involved in cell cycle check-point (PP2A, NT5 and SUPTH), oxidative stress response (GPX1), heat shock proteins production (HSPB1, HSPD1 and HSF2) or by repressing genes involved in antigen recognition (HLA-C and HLA-DBQ1), (Table 2).

Now we have a panel of genes candidate to be a target of cellular response to cosmic radiation, opening interesting perspectives for utilization of DNA microarrays technology as extremely sensitive tool for biodosimeter and cell physiology monitoring in space missions. Further flights and independent analytical methods (Northern blot, RT-PCR) will be required to confirm the significance of these results

Table 2 - Modulation genes group

Cellular checkpoint (PP2A, NT5, N-RAS)
Oxidized Stress (GPX1)
Heat Stress (HSPB1, HSPD1,HSF2)
Ag Recognition (HLA-C,HLA-DBQ1)
Cytokines, cellular morphology (MLC-2)
Repair control (MSH-2, SUPTH)

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