Dichotomal effect of space flight-associated microgravity on stress-activated protein kinases in innate immunity

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Space flight strongly moderates human immunity but is in general well tolerated. Elucidation of the mechanisms by which zero gravity interacts with human immunity may provide clues for developing rational avenues to deal with exaggerated immune responses, e.g. as in autoimmune disease. Using two sounding rockets and one manned Soyuz launch, the influence of space flight on immunological signal transduction provoked by lipopolysaccharide (LPS) stimulation was investigated in freshly isolated peripheral blood monocytes and was compared to samples obtained from on-board centrifuge-loaded 1 g controls. The effect of microgravity on immunological signal transduction is highly specific, since LPS dependent Jun-N-terminal kinase activation is impaired in the 0 g condition, while the corresponding LPS dependent activation of p38 MAP kinase remains unaffected. Thus our results identify Jun-N-terminal kinase as a relevant target in immunity for microgravity and support using Jun-N-terminal kinase specific inhibitors for combating autoimmune disease.
gram negative bacteria. Its recognition by TLR-4 forms an important during space flight. LPS is a relatively invariant surface structure of peripheral blood monocytes with LPS in 0 to investigate the effect of stimulation of primary isolated human system with spaceflight and microgravity in particular, we decided Experimental design

Results

Figure 1 | Summary of the experimental procedure and the timeline. The experiment was repeated three times. The first experiment was performed as part of the 2004 Soyuz TMA-4 campaign. Under the name Kappa it was executed during a manned flight to the International Space Station. The experiment was repeated in 2005 during the Maser 10 mission (Amuse) and again in 2012 during the Maser 12 mission (Micimmun). Both missions used sounding rockets to carry the experiment into space. During a parabolic flight the rocket reaches an altitude of approximately 260 km allowing for 5 to 6 minutes of microgravity. The experiments are performed in automated climate chambers termed Flush Units. The Flush Units are placed within the BIM module. The module regulates ambient temperature aboard the rocket and provides electricity to the Flush Units during flight. As an onboard control the Flush Units can be placed in a centrifuge which spins at a speed equal to the force of gravity. The other units are placed on a static rack and are allowed to experience microgravity. Prior to the flight the monocytes are placed inside a rectangular chamber containing two glass slides to which the monocytes attach. At the start of microgravity a timed mechanism releases a spring plunger which replaces the medium in the culture chamber with medium containing LPS. At the end of microgravity a similar mechanism replaces the LPS containing medium with fixative. We would like to acknowledge NASA and Dutch Space for the use of their illustrations in this figure.

Stimulations were performed within 10 hour after blood was drawn. After recovery of the module, the material was investigated for space flight and microgravity-induced effects on LPS-induced signal transduction.

Activation of p38 map kinase is not affected by space flight-associated microgravity. One of the most pronounced biochemical changes following LPS challenge, is the phosphorylation and thereby activation of p38 MAP kinase. Inhibition of p38 MAP kinase protects against endotoxemia in LPS challenged healthy volunteers, although clinical implementation of pharmacological p38 MAP kinase inhibition has not proven viable because of side effects and excessive vulnerability towards bacterial infection. Various genes, like PRKCA, whose expression is known to be exquisitely sensitive to microgravity are known to be regulated by p38 MAP kinase. Hence, it is conceivable that activation of p38 MAPK is impaired during microgravity. As expected, the onboard 1 g control displayed p38 MAP kinase activation (Figure 2). In contrast, activation of p38 MAP kinase was not impaired in microgravity-exposed monocytes, if anything p38 MAP kinase activation appears to be increased. Thereby suggesting that space flight-associated microgravity does not interfere with LPS-induced pro-inflammatory signal transduction.

Jun-n-terminal kinase is not activated by lps during space flight-associated microgravity. In general inflammatory stimuli co-activate p38 MAP kinase and Jun-N-terminal kinase, as activation of both kinases displays similar kinetics. Nevertheless, they are activated by different upstream kinases (MKK3/6 and MKK4/7 for p38 MAP kinase and Jun-N-terminal kinase, respectively). Thus, at
least theoretically, the possibility exists that space flight-associated microgravity targets Jun-N-terminal kinase without a concomitant effect on p38 MAP kinase. Surprisingly, during microgravity the capacity of LPS to stimulate Jun-N-terminal kinase activation is lost, whereas monocytes stimulated with LPS during the onboard control condition were completely Jun-N-terminal kinase activation proficient (Figure 3). Thus, microgravity evokes a dichotomy in immunological signaling, allowing p38 MAP kinase activation to proceed, but incompatible with LPS dependent Jun-N-terminal kinase activation. Although it does not involve immunologically relevant cells, the observation that the induction of c-Jun in A431 cells by epidermal growth factor is absent in microgravity26, fits well with our findings. Especially since it has been well established that Jun-N-terminal kinase is involved in the induction of c-Jun and p38 MAPK is not.

Effects of space flight-associated microgravity on erk activation. Although less pronounced than the effect on Jun-N-terminal kinase, an inhibitory influence of microgravity on the capacity of LPS to provoke activation of the extracellular regulated kinase (ERK) was observed (Figure 4). This corresponds with recent findings in T cells that show a minor effect of microgravity on ERK phosphorylation in unstimulated T cells27. Thus, the effect of microgravity is not solely restricted to Jun-N-terminal kinase.

Diminished activity of the immune system is among the foremost effects of space flight on the human body28. Importantly, this diminished activity of the immune system is well-tolerated. Over forty years of experience with manned space flight have now conclusively demonstrated that man can easily survive and work in weightless conditions, despite chronic repression of the body’s immune functions29. This suggests that mimicking space-related immunosuppression on earth may be a fairly safe way of suppressing the action of the immune system. Although experimental requirements allowed for only 6 minutes of microgravity, there is good evidence that even a relatively short inhibition of kinases can have a substantial effect on functionality of the innate immune system30. Therefore we feel that the observed effect on Jun-N-terminal kinase is relevant to the explanation of space flight-associated immunosuppression. The observed dichotomy between p38 MAP kinase and Jun-N-terminal kinase is consistent with these observations: p38 MAP kinase is important for fighting immediate bacterial threats and thus its inhibition causes undesirable side effects in these and other respects31. On the other hand, Jun-N-terminal kinase is more associated with chronic inflammatory responses32,33 whose inhibition represents a clear and for now unresolved clinical need.

Methods

Microgravity experimentation. This study received Institutional Review Board approval from the Academic Medical Center and informed consent was obtained in accordance with the Declaration of Helsinki. The methods were carried out in accordance with the approved guidelines. Blood was drawn from young healthy volunteers (health status was ascertained by a physical examination) in Vacutainer tubes (Becton-Dickinson, Breda, the Netherlands) containing EDTA-K3. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of healthy volunteers using standard density gradient centrifugation over Ficoll-Paque Plus (Amersham Biosciences AB), followed by washing and resuspension in IMDM supplemented with 10% fetal bovine serum, penicillin, streptomycin, and amphotericin. Blood was drawn ten hours before launch time. The experiments were performed in the “Biology In Microgravity” flight module that has been developed by Dutch Space (Leiden, the Netherlands) and the Kubik module developed by ESA.
Figure 3 | LPS-dependent Jun-N-terminal kinase activation is sensitive to space flight-associated microgravity. The figure shows phospho-Jnk analysis of LPS stimulated monocytes in the presence (1 G) or relative absence of gravity (0 G). Fixed monocytes were stained fluorescently (green) and the nuclei were visualized by DAPI (blue) (Figure 3A). Comparison between individual cells clearly shows a brighter staining when stimulated with LPS in the presence of gravity. Staining intensity was measured at different exposure times (Figure 3B) and mean fluorescence was finally determined at 2000 ms (Figure 3D). Microgravity significantly impairs the activation of Jnk in monocytes. Analysis of nuclear localization of activated Jnk shows no change (Figure 3C). The graphs are based on 32 determinations. *P < 0.05.

Figure 4 | Effects of microgravity on ERK activation. The figure shows phospho-Erk analysis of LPS stimulated monocytes in the presence (1 G) or relative absence of gravity (0 G). Fixed monocytes were stained fluorescently (green) and the nuclei were visualized by DAPI (blue) (Figure 4A). Comparison between individual cells clearly shows a brighter staining when stimulated with LPS in the presence of gravity. Staining intensity was measured at different exposure times (Figure 4B) and mean fluorescence was finally determined at 2000 ms (Figure 4D). Microgravity impairs the activation of Erk in monocytes. Analysis of nuclear localization of activated Erk shows no change (Figure 4C). The graphs are based on 32 determinations.
(Noordwijk, The Netherlands), which were loaded with automated experimental container units developed by the Centre for Concepts of Mechatronics (Nuenen, The Netherlands). Launch vehicles were sounding rockets developed by Swedish Space Corporation (SSC) and the Soyuz-TMA rocket produced through the Russian space program. Experiments employed a late access unit, which was installed into the payload 4 hours before launch. An integrated thermal controller maintained the experiment temperature at 36.5 °C. Monocytes, a 100 ng/ml LPS containing activation solution and 4% formaldehyde fixative in PBS were loaded into the flight hardware. The hardware devices contain two experimental units of which each unit contains three chambers. The two outer chambers were connected via small tubes in the lid with the middle chamber which contained the biological material. The chambers for the fixative contain special storage compartments, since fixatives for biological material diffuse easily through rubbers and plastics. Electronically-controlled springs released activation solution and fixative at pre-set time points, so that the solutions in the storage chambers are pushed via the connecting tubes into the reaction chamber. The LPS-containing solution or the vehicle control was transferred shortly after the onset of microgravity, whereas the fixative was transferred exactly 5 min later. Technical failure was a substantial problem: of the 96 biological experiments send in space only 31 samples obtained from the Maser 12 flight qualified for further analysis, limiting the number analyses possible. Realistic vibration tests with the BIM-2 module were performed at the Environmental Test Laboratory of Thales Cryogenics (Eindhoven, The Netherlands) during flight qualification procedures and did not affect experimental outcomes (not shown). Accelerations of the payload and of the centrifuge were continuously monitored and recorded during flight.

Analysis of kinase activation. Following recovery, cells were extensively washed with PBS and permeabilized with PBS containing 0.1% Triton X-100. Subsequently, samples were incubated overnight at 4 °C with anti-phospho-p38 MAPK (Thr180/ Tyr182) monoclonal rabbit antibody, anti-phospho-Jun-N-terminal kinase (pThr183/tyr185) monoclonal rabbit antibody or anti-phospho-Erk1/2 (pThr202/pTyr204) monoclonal rabbit antibody, all obtained from Cell signaling Technology (http://www.cellsignal.com/) followed by incubation with the secondary antibody anti-rabbit AlexaFluor488 (Invitrogen, Carlsbad, CA) for 1 h at room temperature and counterstained with DAPI. Confocal images were obtained on a Leica TCS SP2 unit containing three chambers. The two outer chambers were connected via small tubes in the lid with the middle chamber which contained the biological material. The chambers for the fixative contain special storage compartments, since fixatives for biological material diffuse easily through rubbers and plastics. Electronically-controlled springs released activation solution and fixative at pre-set time points, so that the solutions in the storage chambers are pushed via the connecting tubes into the reaction chamber. The LPS-containing solution or the vehicle control was transferred shortly after the onset of microgravity, whereas the fixative was transferred exactly 5 min later. Technical failure was a substantial problem: of the 96 biological experiments send in space only 31 samples obtained from the Maser 12 flight qualified for further analysis, limiting the number analyses possible. Realistic vibration tests with the BIM-2 module were performed at the Environmental Test Laboratory of Thales Cryogenics (Eindhoven, The Netherlands) during flight qualification procedures and did not affect experimental outcomes (not shown). Accelerations of the payload and of the centrifuge were continuously monitored and recorded during flight.

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