From Space to the Patient: 
A new cytokine release assay to monitor the immune status of HIV infected patients and sepsis patients

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BACKGROUND
Monitoring of humans either in the healthy men under extreme environmental stress like space flight, in human immunodeficiency virus (HIV) infected patients or in sepsis is of critical importance with regard to the timing of adequate therapeutic (counter-)measures. The in vivo skin delayed-type hypersensitivity test (DTH) served for many years as a tool to evaluate cell mediated immunity. However, this standardised in vivo test was removed from the market in 2002 due to the risk of antigen stabilization. To the best of our knowledge an alternative test as monitoring tool to determine cell mediated immunity is not available so far. For this purpose we tested a new alternative assay using elements of the skin DTH which is based on an ex vivo cytokine release from whole blood and asked if it is suitable and applicable to monitor immune changes in HIV infected patients and in patients with septic shock.

METHOD & RESULTS
Two pilot studies including either outpatients with HIV infection (n=9; study name: DTH@HIV) and critical ill patients with septic shock admitted to intensive care (n=78, study name: HoSpace) at a large academic hospital in Munich were performed. Ethical approval and informed consent was given by the patients or the legal representatives.

DTH@HIV: Nine patients with HIV infection were divided into 3 groups: HIV-infected subjects on highly active antiretroviral therapy (HAART) with a CD4+ cell count >350/µL (group I) or a CD4 cell count <350/µL (group II) and HIV-infected HAART-naive subjects with a CD4+ cell count >350/µL (group III). All groups were compared with healthy volunteers (n=3).

HoSpace: 75 patients in condition of septic shock as defined by the members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee, admitted to the intensive care units of the Department of Anesthesiology, University Hospital Munich, Germany, were enrolled into this clinical observational trial. Pregnant women, patients with hemorrhagic shock, burn injury, organ transplantation or glucocorticoid treatment were excluded. Blood was drawn within 24 hours (baseline = B) and on days 4, 7 and 10 after onset of sepsis.

The ex vivo cytokine release assay was performed in a three step process: (1) blood collection, (2) whole blood ex vivo incubation over 48 hours without or with a standard set of well-defined recall antigens as comparable to those used formerly in the skin delayed type-hypersensitivity (DTH) test, (3) interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)-determination from the assay supernatant by Luminex®xMap technology.

Our evaluations showed that the IL-2, IFN-γ and TNF-α release from whole blood can be induced by a standard set of well-defined stimuli derived from the skin DTH test, thereby gradually reflecting immunological response. Additionally, the assay revealed a strong and significant correlation of the cytokine concentrations with the HIV group classifications and course of septic disease.

CONCLUSION:
In summary, the new cytokine release assay as evolved for space applications might be a suitable diagnostic tool of immune monitoring in either HIV infected patients as well in septic patients. Future studies in larger patient cohorts are warranted to determine the value of this cytokine release assay for prediction of long-term survival and sequelae.

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