INFLUENCE OF PVA COATED NANOPARTICLES ON SURVIVAL AND FUNCTIONALITY OF HUMAN IMMUNE CELLS

Cindy Strehl[1, 2], Timo Gaber-Elsner[1, 2, 3], Markus Wagegg[1, 2], Manuela Jakstadt[1,2,3], Martin Hahne[1, 2, 4], Saskia Schellmann[1,2], Barbara Esche-Szostak[1]; Géraldine Coullerez[5]; Heinrich Hofmann[5]; Gerd-Rüdiger Burmester[1], Frank Buttgereit[1]

[1]Department of Rheumatology and Clinical Immunology, Charité University Medicine (CCM), 10117 Berlin, Germany;
[2]German Rheumatism Research Centre (DRFZ), 10117 Berlin, Germany;
[3]Berlin-Brandenburg Center for Regenerative Therapies, Charité University Medicine (CVK), 13353 Berlin, Germany
[4]Berlin-Brandenburg School for Regenerative Therapies, Charité University Medicine (CVK), 13353 Berlin, Germany
[5]École polytechnique fédérale de Lausanne EPFL, Institute of Materials Powder Technology Laboratory, Lausanne, Switzerland

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Background: Nanotechnology has developed into a key technology of the 21st century. Over the recent years, the number of nanotechnical products has received an enormous boost. More and more efforts are being done to use this technology in human medicine for diagnostic and therapeutic purposes. Therefore, crucial questions concern the safety aspects. The focus of our work here was to identify possible effects of nanoparticles on human immune cell function.

Objectives: We analysed clinical relevant interactions between PVA coated nanoparticles (spions) and human immune cells.

Methods: 100µl of whole blood obtained from patients with rheumatoid arthritis (RA) or healthy donors were incubated with 100µl serum free RPMI 1640. Functionalised spions were added at varying concentrations, and cells were incubated for 24h. After lysis of erythrocytes, cells were stained for apoptosis and necrosis using Annexin V and 7AAD, respectively. Samples were analysed by flow cytometry. As a second approach, PBMCs were isolated from blood samples of healthy donors and RA patients, and CD4 positive T cells were separated via MACS. T cells were incubated with/without PHA and/or with/without PVA spions at different concentrations. Activation (CD25 expression) of cells was analysed by flow cytometry. Functionality was determined via proliferation measurements of CFSE (carboxyfluorescein diacetatesuccinimidyl ester) labeled T cells after 72h under normoxic (5% CO_2 and 18% O_2) or hypoxic (5% CO_2 and <1% O_2) conditions by flow cytometry.

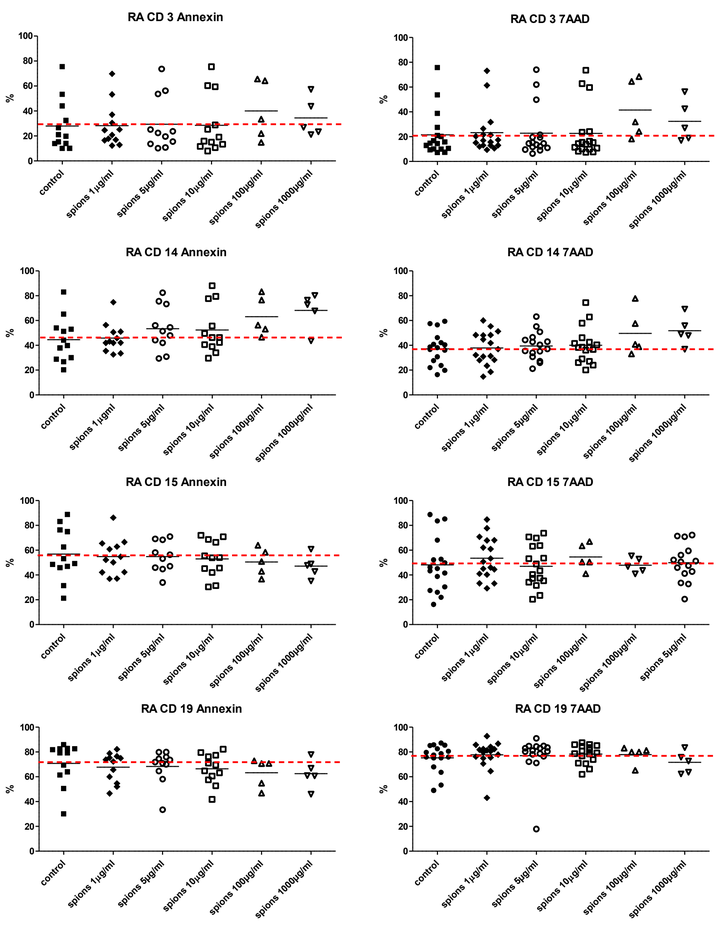

Figure 2: Influence of PVA coated nanoparticles (spions) on the survival of human immune cells. Whole blood survival analysis was performed with blood samples obtained from **RA patients**. Cells were analysed for specific surface markers, Annexin V (apoptosis marker) and 7AAD (necrosis marker). There is no measurable influence of varying spion concentrations on the frequency of Annexin V or 7-AAD positive cells.

Results: Altogether, blood samples from 18 healthy donors and 19 patients suffering from RA were analysed for induction of apoptosis and necrosis in different cell types (figure 1 & figure 2). The results on cell survival did not demonstrate any short-term general toxicity of PVA spions at concentrations less than 1000µg/ml on the several different blood cell subsets examined. Furthermore, T cells were isolated from 14 healthy donors and 19 RA patients for functional analysis (figure 3). There is no influence of PVA spions on T cell activation and proliferation at concentrations less than 1000µg/ml.

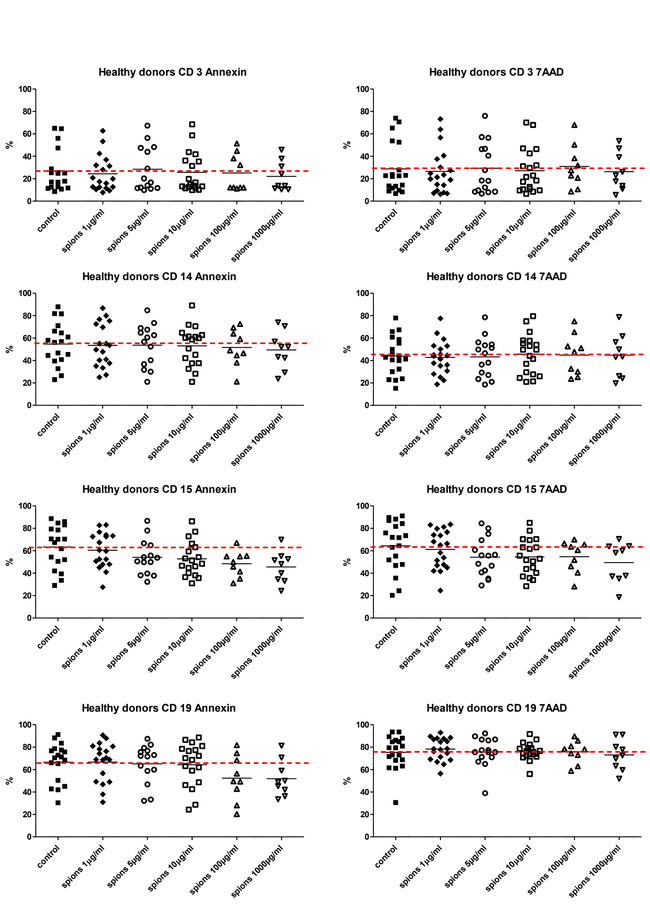

Figure 1: Influence of PVA coated nanoparticles (spions) on the survival of human immune cells. Whole blood survival analysis was performed with blood samples obtained from **healthy donors**. Cells were analysed for specific surface markers, Annexin V (apoptosis marker) and 7AAD (necrosis marker). There is no measurable influence of varying spion concentrations on the frequency of Annexin V or 7-AAD positive cells.

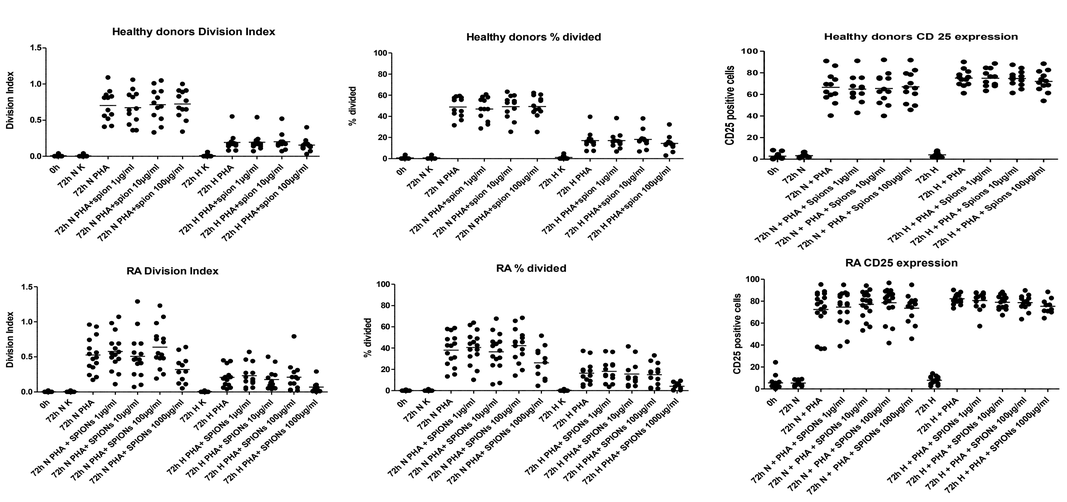

Figure 3: Proliferation and CD25 expression of isolated CD4+ T cells obtained from healthy donors and RA patients treated with varying spion concentrations (1-1000 µg/ml). No significant effects of PVA coated spions on the proliferation, the ability to become activated and activation of CD4+ T cells could be shown.

Conclusion: PVA coated nanoparticles at concentrations up to 1000µg/ml (i) do not increase the frequencies of apoptotic or necrotic human immune and (ii) do not impair crucial functional activities of human T cells such as activation and proliferation.

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