Are inter-individual immune responses a challenge for the standardisation of immunogenicity in vitro tests?

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INTRODUCTION

A number of guidance documents and guidelines across industrial sectors have been developed with the aim to predict changes in the immune function after exposure to xenobiotics. However, the complexity of the human immune system, interspecies variations and even fluctuations in inter-individual responses make the assessment of adverse effects of products challenging.

In this study, we have investigated the variability of the complement activation of healthy donors and patients suffering from cancer, immunodeficiency and allergies after exposure to empty liposomes. Liposomes are well-known delivery systems for e.g. anti-cancer drugs and recently received attention as vectors for SARS-COV2 vaccines. Nevertheless, tiny changes of their physicochemical properties can trigger adverse effects such as complement activation related pseudoallergy (CARPA). Ongoing standardisation efforts for assessing the activation of the complement system in vitro are confronted with a significant variability of the immune responses. This observation requires in-depth investigations, since an underprediction of the immunogenicity of the liposomal products can have severe consequences for patients.

METHODOLOGY

We have optimised an existing protocol for the quantitative determination of a complement cleavage product (iC3b protein) by systematically evaluating various critical assay parameters including positive/negative controls, incubation times etc. using commercially available pooled serum from healthy donors. The generation of iC3b was measured with a commercial Enzyme-linked Immunosorbent Assay kit (cat A006, Quidel). Three liposomes with different physical chemical properties (FormuMax Scientific, Inc. (USA)) were tested by using serum from healthy donors. The effect of liposomes on iC3b was measured in human serum samples categorised according to their blood types, gender and health status. The liposome with the highest iC3b induction rate was tested in serum of 56 patients in total (in.vent, Germany) suffering from cancer, allergies and immunodeficiencies. Furthermore,

individual components of the liposome including polyethylene glycol (PEG), cholesterol (CHOL) as well as hydrogenated soybean phosphatidylcholine (HSPC) were tested.

RESULTS

The iC3b induction potential of 3 different liposomes and PEGylated liposomal doxorubicin hydrochloride was compared to a number of positive controls with different potencies. The assay demonstrated a good reproducibility between independent biological runs. In particular, a negatively charged liposome as well as its individual constituents (PEG and HSPC) induced iC3b cleavage in pooled serum of healthy donors. To better understand the biological relevance of a pooled serum to predict individual responses, we investigated the serum of 32 healthy donors and we observed a significant variability (from 1.5±0.53 to 2.46 ±0.44 fold-change as compared to the negative control). We then analysed the serum of 56 patients suffering from cancer, immunodeficiency and allergies. We observed a clear correlation between diseases and the level of complement activation, while blood types and gender seemed not to play a role. In particular, as compared to healthy donors, patients suffering from cancer and allergies showed a 4.16 and 1.36-fold increase of iC3b levels, respectively, whereas the complement system of immunocompromised patients was significantly decreased.

DISCUSSION

The human immune system significantly differs from animal systems but it is also highly variable between individuals because of heritable and non-heritable influences. If not properly addressed, inter-individual variability can have a significant impact on the predictive capacity of in vitro assays designed to identify changes in the immunological processes after chemical exposure.

By using liposomes, well-known for their potential to activate the human complement system, we could demonstrate the considerable difference of immune responses in an in vitro assay between healthy donors. Furthermore, we obtained a first trend suggesting an increase of complement activation in patients suffering from allergies and cancer whereas patients with immunodeficiencies showed decreased reactions.

These results call for systematic analyses on the impact of inter-individual variations when responding to stressors. In longer terms, a new research branch aiming to advance personalised risk assessment methodologies is needed, which would allow for characterisation of the biological relevance of human based in vitro methods while supporting the identification and protection of susceptible individuals.