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Data sieving analysis as a novel method to asses immunotoxic exposure to dioxins retrospectively

Sir,

TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is a chemical compound which strongly activates the endogenous transcription factor AHR and thus mediates severe reproductive and developmental problems, immune system damage, and interference with regulatory hormones. An important, yet difficult to asses, aspect of TCDD-immunotoxicology are the long-term effects of exposure in the ageing population. The use of biomarkers of exposure from blood or other easily available tissues has the potential to overcome costly and invasive measurements of body dioxin burdens, and at the same time gives information on the biological/immunotoxic efficiency of the compound. In long-term studies it might even be possible to assess exposure retrospectively, i.e. when damage or adverse effects persist after elimination of the toxic compound. For dioxins and related polyhalogenated aromatic hydrocarbons, simple, valid and robust biomarkers of immunotoxicity have not been found so far, albeit numerous attempts have been made in identifying relevant shifts in blood leukocyte frequencies. In this context, we used multidimensional analysis of flow cytometric data with a novel data sieving approach. The goal of data pattern classification concerns the exhaustive knowledge extraction from all available flow cytometric (single cell molecular profiling) parameters by the determination of the most discriminatory data patterns. The technique was successful in the identification of mechanisms and therapeutic success in complex diseases like allergies [1]. In a previous study we had determined frequencies of lymphocyte subpopulations (CD3, CD4, CD8, CD14, CD19, CD56, CD57, HLA, forward scatter, side scatter) from 10 workers exposed to high doses of 2,3,7,8-TCDD and other dioxins 20 years ago, and from 8 unexposed control persons. In vitro data had revealed a persistent damage of T cells in these workers [2], but the analysis for frequency changes of known subpopulations identified by T-cell, B-cell or NK-cell markers did not yield any significant differences between exposed workers and controls. We now re-analysed the flow cytometric data from that study with the CLASSIF1 computer programme for data pattern analysis. We found that dioxin-exposed persons exhibited a discriminatory data pattern of increased forward scatter of $CD56^+$ lymphocytes, of increased CD14 on $CD14^+$ $CD56^+$ lymphocytes and of increased percentage of $CD19^+$ lymphocytes as compared to unexposed workers with positive and negative predictive values of 100% and 90.9% (see Fig. 1). These three parameters would have been hardly considered jointly under a deductive working hypothesis. The finding of differences in CD19 and CD56 cells is, however, in agreement with observations regarding B-cell and NK-cell frequency after PCB or dioxin exposure [3]. The validation of this biomarker profile as robust biomarkers of exposure requires the classification of higher numbers of exposed and non-exposed persons, partly in a blinded fashion, despite the fact that CLASSIF1 data sieving is comparatively resistant against the classification of random data patterns as biologically meaningful [4]. As flow cytometric data of exposed versus unexposed persons were generated in many research teams, for either dioxin or other immunotoxic compounds, it would be feasible to re-analyse them with CLASSIF1. We would be interested in applying cytomics to this wealth of information together with interested colleagues.

In conclusion, we have, for the first time, used data sieving for determining immunophenotype profiles as biomarker of exposure in dioxin-exposed workers, and identified dioxin-specific combinations of lymphocyte

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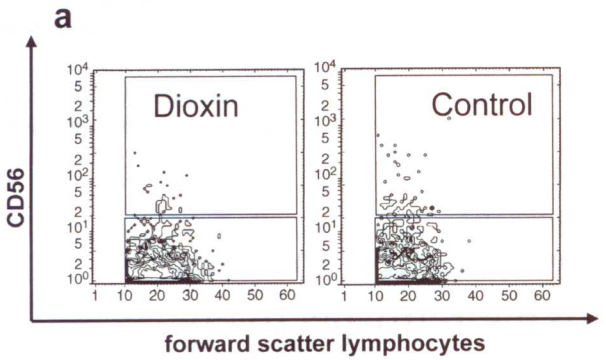

Fig. 1. a): 177 list mode data files from two data sets from cells analysed in a FACScan™ flow cytometer (Becton-Dickinson, Mountan View, U. S.A.) (2) were classified with the self-learning programme CLASSIF 1 (4). Lymphocytes were gated automatically and for each data file the respective fluorescence data evaluated individually according to fluorescence intensity. Shown are two typical contour plots, one from data of a control person, another from data of a dioxin-exposed person. The programme classified parameters as predictive, even though they are not visible for the eye or by deductive measures. b): Classification of control (C) and dioxin-exposed persons (D) using the three discriminatory patterns found in the analysis of the list mode data files. With two exceptions (person 2) all control persons and all dioxin persons were identified by the programme, when the discriminant parameters were used as a mask. Black box: changed; white box: unchanged; C,D, *c,d* control or dioxin-exposed person, as known (bold letters) or classified by CLASSIF 1 (italics). All dioxin-exposed persons showed changes in at least one of the discriminators (CD19, FSC/CD56, CD14/CD56), only two control persons displayed one or two such changes. Thus, the learning mode of the programme yielded discriminators, which, however, need to be validated further on unknown samples.

surface markers at the individual person level in a preliminary way. We propose cytomics as a powerful tool in assessing post hoc effects of exposure, even years after first exposure to immunotoxic compounds.

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