**Improving the in vitro assessment of sensitizing chemicals by an activation-induced marker (AIM) assay: a study on p-phenylenediamine and Bandrowski's base**

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The hair dye p-phenylenediamine (PPD) and its oxidation product Bandrowski´s base (BB) can trigger allergic contact dermatitis (ACD), a T cell-mediated skin disease, in susceptible individuals. Currently there is no validated in vitro test for predicting chemical-specific T cell activation. Therefore, we aim to establish a short-term activation-induced marker (AIM) assay to detect chemical-specific T cells in human peripheral blood mononuclear cells (PBMC). Using flow cytometry, we performed PPD and BB concentration series to control for potential fluorescence interference, chemical toxicity, as well as monocyte (antigen-presenting cells) and T cell function.

We determined an optimal working concentration for both substances as 6 µM, considering the absence of flow cytometry interferences and toxic effects in T cells. Both chemicals induced an upregulation of T cell receptor (TCR) specific surface activation markers (CD154 and CD137) on CD4+ and CD8+ memory T cells, respectively, with higher frequencies of chemical-specific T cells among allergic individuals. Pilot data on TCR sequencing revealed extensive T cell cross-reactivity between PPD and BB, indicating that PPD and BB-induced epitopes appear structurally related.

Collectively, AIM assays can be used to identify and quantify PPD and BB-specific T cells with high sensitivity. AIM assays may overcome current limitations of in vitro chemical allergy diagnosis and predictive sensitizer testing, also contributing to the risk assessment of chemical sensitizers.