The Application of an Improved Lymphocyte Transformation Test to Diagnose non-immediate Drug Hypersensitivity Reactions

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Background

Diagnosing non-immediate drug hypersensitivity reactions (DHR) is primarily based on a detailed history and clinical findings at the time of reaction. Skin and drug provocation tests can further support the diagnosis. However, skin and drug provocation tests carry a risk of inducing severe systemic reactions. The standard lymphocyte transformation test (LTT) is an *in vitro* test that determines the proliferation of drug-specific immune cells upon stimulation with the suspected drug(s). This investigation aimed to establish and improve the *in vitro* LTT blood test to give information about proliferation- and cytokine profiles of drug-specific immune cells.

Methods

Eighteen patients with suspected non-immediate DHR were included in the study. Peripheral blood mononuclear cells (PBMCs) were isolated from blood and stained with carboxyfluorescein succinimidyl (CFSE). The PBMCs were stimulated with phytohemagglutinin (10 µg/ml) and tetanus toxoid (10 µg/ml) as positive controls and four drugs at three concentrations: amoxicillin (250, 25, 2.5 µg/mL), ampicillin (250, 25, 2.5 µg/mL), dicloxacillin (62.5, 6.25, 0.625 µg/mL) and penicillin G (1000, 100, 10 µg/mL) for seven days. On day seven, the cells expression of proliferation- and cell-type-specific markers (CFSE, CD3, CD4, CD8, CD56) in addition to their production of cytokines (IL-2, IL-5, IL-8, IL-13, IL-17A, IL-22 and IFN-γ) were analysed by flow cytometry.

Results

Non-toxic concentrations of amoxicillin, ampicillin, dicloxacillin, and penicillin G were identified, and the flow cytometer-based LTT test was successfully established. A trend of higher frequencies of drug-specific T cells expressing the markers of T Helper T cells (CD4), memory (CD45RO) and one or more of the cytokines IL-5, IL-13, IL-17A, IL-22 and IFN-γ were observed towards the culprit DHR suspected drug. It also became evident that memory T helper cells, rather than NK- or cytotoxic cells, were by far the most prevalent drug-specific cell type. The LTT data also show a generally positive correlation between drug concentration level exposure and PBMC reaction severity.

Conclusion

Altogether, the results show that the *in vitro* flow cytometric LTT test may be a useful method for identifying the culprit drug in non-immediate drug hypersensitivity reactions. More data, including tests of healthy control subjects, are needed to describe the sensitivity and specificity of the LTT test.