In vitro method for the identification of DNP- and TNP-specific T cells in the naive compartment of human blood

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Introduction

In the past commonly used methods for the identification of sensitizers involved animal testing. This approach has to be urgently replaced by in vitro methods for ethical and regulatory reasons. We developed a new method for the identification of human naive T cells with a certain antigen specificity, including chemical-specific T cells. We used DNP and TNP as models for semantically chemicals.

Material and methods

Monocytes were isolated from PBMC of healthy donor using CD14 Microbeads. An aliquot of purified monocytes was cryopreserved for later use. The CD14+ monocytes were differentiated into DCs in the presence of IL-4, GM-CSF, TNF-α, and IL-12. The generated DCs were subsequently modified by resuspending cells in 1 mL of a 4 mM DNBS or 3 mM TNBS stock solution. The T cell priming was set up using 10⁷ T cells, 10⁶ DCs, and 5x10⁶ feeder cells and CD8+ T cells were cultured using RPMI 1640 + 5% AB-serum. For priming, T cells were harvested on day 8, washed and reconstituted with modified DCs. Cytokine expression by T cells was analyzed by flow cytometry after intracellular staining.

Summary

In summary, we established a fast and robust in vitro assay to identify contact sensitizer-primed T cells. There are still some issues to be solved for the development of an assay that allows the prediction of the allergenicity of a particular chemical in the human population without the use of murine DCs. Hence, we replaced TNP-modified MoDCs with TNP-modified fastDCs for priming of naive T cells and restimulation, which allowed us to identify TNP-specific CD4+ and CD8+ T cells, establishing fastDCs as competent APCs. Moreover, due to the shorter culture period, the viability and recovery of fastDCs is increased compared to MoDCs and cryopreservation of T cells to the priming culture is not required.

Results

Identification of DNP- and TNP-specific T cells

DNP- and TNP-specific T cells were detectable in all samples tested from three donors for each specificity, using the protocol described in figure 3A. TNP-reactive and TNP-specific T cells from CMV-infected donors with soluble CMV IE-1 protein or pulsed fastDCs or MoDCs showed that both fastDCs and MoDCs were able to effectively activate IFN-γ-specific CD8+ T cells, whereas monocytes did not induce T cell activation. This result indicates that both fastDCs and MoDCs cross-present exogenously delivered protein antigens into the MHC class-I pathway, leading to the activation of CD8+ T cells.

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Cytokines for T cell priming and expansion culture

Since not all chemicals may induce IFN-γ production, we included the analysis of other effector cytokines (TNF-α, IL-17, and IL-22). So far we did not find IL-17 or IL-22 expression in TNP-primed and restimulated cell cultures, but TNF-α was found to be detectable in all tested samples. Co-expression analysis of TNP-specific CD8+ T cells showed that cells reacting to TNP- as well as DNP-modified fastDCs or MoDCs were not detectable.

In summary, we established a fast and robust in vitro assay to identify contact sensitizer-primed T cells. There are still some issues to be solved for the development of an assay that allows the prediction of the allergenicity of a particular chemical in the human population without the use of murine DCs. Hence, we replaced TNP-modified MoDCs with TNP-modified fastDCs for priming of naive T cells and restimulation, which allowed us to identify TNP-specific CD4+ and CD8+ T cells, establishing fastDCs as competent APCs. Moreover, due to the shorter culture period, the viability and recovery of fastDCs is increased compared to MoDCs and cryopreservation of T cells to the priming culture is not required.

Furthermore, we examined the suitability of the activation marker CD137 as a readout, as this may allow the identification of antigen-specific cells irrespective of the expression of effector cytokines. However, CD137 expression was in part independent of the restimulation with or without antigen, excluding CD137 as a marker for the identification of antigen-specific T cells (fig. 3B). We therefore recommended the combined detection of IFN-γ and TNF-α in intracellular cytokine staining as readout system.

Reference