GENERATION OF MULTI-FUNCTIONAL AND TUMORICIDAL NKT-LIKE ANTIGEN-SPECIFIC T CELLS

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Background Natural Killer T cells (NKTs) offer great promise for cancer immunotherapy. However, NKTs are extremely rare within human peripheral blood, posing difficulties to isolation and ex vivo expansion. Moreover, native NKTs may develop immunosuppressive activity within the tumor microenvironment.1 For these reasons, in vitro generated CD56+ T cells (NKT-like cells) have emerged as an alternative to native NKTs.2 However, as CD56+ T cells are in vitro generated from precursors (e.g., PBMCs) mostly using non-antigen specific stimulants (e.g., IL-15, IFN-γ, PMA/ionomycin, or PHA), tumor killing capacity of those induced CD56+ T cells has been limited to non-antigen specific killing pathway. Moreover, questions remain unanswered regarding how best to enhance productivity and cytotoxic capacity of CD56+ T cells.

We demonstrate in vitro generation of antigen-specific CD56+ T cells from human antigen-specific T cells (ASTCs) with a comparison of phenotype and cytotoxic functions between in vitro derived NKT-like cells and NKTs isolated from human apheresis.

Methods During in vitro production of ASTCs (CD3+CD8+), wherein antigen specificity is created by TCR stimulation with a single antigen peptide (MART-1, ELAGIGILTV), a subset of CD56+ T cells was generated. CD56+ and CD56- T cell subsets were isolated using immunomagnetic separation. Phenotypic and functional characterization of CD56+ and CD56- populations from ASTCs were compared to NKTs directly isolated from human apheresis.

Results MART-1-specific ASTCs were in vitro generated through scaled-up production to produce at least 700 million cells from a single donor batch and CD56+ T cell population increased from 4.49% in starting apheresis to 43% out of total ASTCs. Interestingly, both CD56+ and CD56- subsets exhibited similar MART-1 tetramer positivity of 88%. During co-culture of ASTCs with antigen peptide-loaded T2 cells, CD56+ subset induced greater T2 cell death than the CD56- subset, while greater IFN-γ secretion was detected from the CD56- subset (figure 1). In tumor cell killing assay with MeWo cells, all three effector cell populations (total MART-1-specific ASTCs, CD56+, CD56- cells) induced tumor killing efficiently, with up to 50% cell death observed with a 10:1 effector to target ratio (figure 2).

Conclusions Antigen-specific CD56+ T cells were generated and confirmed to have cytotoxic potential comparable to CD56- T cells in antigen-specific and MHC-restricted manner. To further understand generation and function of NKT-like cells, we are investigating generation of CD56+ T cell subset during production of ASTCs specific to additional antigens, and their cytotoxic functions in non-antigen specific and MHC-unrestricted manner.

REFERENCES

Ethics Approval This study was approved by HemaCare – Biorepository Protocol 001, Collection or Procurement of Samples from Healthy Participants Utilizing United States (US), European Union (EU) and Other International Criteria (Pro00034695).

Abstract 928 Figure 1 CD56+ T cells have cytotoxic capacity against MART-1 peptide-loaded T2 cell line at level comparable to CD56- T cells, but IFN-γ secretion from CD56+ T cells was less than CD56- T cells. After total MART-1-specific ASTCs were split into CD56+ and CD56- cells, 20,000 CD56+ or CD56- T cells were plated in a 96-well round-bottom plate with 20,000 T2 cells expressing HLA-A*02:01 alone or in the presence of increasing concentrations of HLA-A*02:01 restricted MART-1 peptide (ELAGIGILTV) or HLA-A*02:01 restricted CMVpp65 peptide (NLVPMVATV) as a negative control. After 18-24 hours, cells and supernatant well were collected. 7-AAD-stained T2 cells were quantified out of total T2 cells for cytotoxicity assay (A-B). Also, IFN-γ concentration from the supernatants was analyzed using Meso Scale Discovery (C-D).
Abstract 928 Figure 2 CD56+ and CD56- T cells from antigen-specific T cell development have comparable tumoricidal capacity. Target cells (MeWo tumor cell line expressing HLA-A*02:01) were pre-plated at 20,000 cells/well. Next day, effector cells (MART-1-specific ASTCs, HLA-A*02:01-restricted) were separated into CD56+ and CD56- cells and added to wells of target cells at 1:1, 5:1, or 10:1 effector:target ratios in triplicate. After 24 hours co-culture, T cells were washed from the adherent tumor cells using PBS. Remaining viable tumor cells were then assessed using Cell Titer Glo assay. Cytotoxicity was calculated as [1-(experimental RLU/target only RLU)*100, where RLU stands for relative luminescent units.

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