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PD-1 and Tim-3 blocking dose not improve the apoptosis of leukemic cells by peripheral blood CD8+ T cells in chronic lymphocytic leukemia

Shakiba Jafarkhani[1]

Abolghasem Ajami[1], Hadi Hossein-Nataj[1], Mohammad Eslami-Jouybari[2,3], Maryam Ghoreishi[2,3], Hossein Asgarian-Omran[1, 2, 4 *]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

2. Gastrointestinal Cancer Research Center, Non-Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran

3. Department of Hematology and Oncology, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran

4. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background:

Patients with chronic lymphocytic leukemia (CLL) have profound defects in function of T-cells developing an exhausted phenotype which is recognized by the expression of multiple immune checkpoint receptors such as PD-1 and Tim-3. In the last decade, immunotherapy strategy based on the immune checkpoint inhibitors has achieved invaluable success in tumor therapy. In this in vitro study, the effect of PD-1 and Tim-3 blocking was investigated to restore the function of exhausted CD8+ T-cells in CLL patients.

Methods:

Peripheral blood mononuclear cells were isolated from 16 patients with CLL and CD8+ T cells were positively isolated using magnetic beads separation method. Isolated CD8+ T cells were treated with either blocking antibodies against PD-1 and Tim-3 and isotype matched control antibodies and then co-cultured with CLL leukemic cells as target cells. Treated CD8+ T cells were stimulated with anti-CD3/CD28 antibodies and recombinant IL-2. The percentage of apoptotic leukemic cells and expression of apoptotic genes (Bax, Bcl-2 and Caspase-3) were evaluated by flow cytometry and Real-Time PCR methods, respectively. IFN-γ and TNF-α concentration was also measured using ELISA.

Results:

Flow cytometric analysis of apoptotic leukemic cells indicated that the blockade of PD-1 and Tim-3 did not significantly improve the cytotoxicity effects of CD8+ T-cells on CLL cells which then were confirmed by gene expression analysis of Bax, Bcl-2 and Caspase-3 which was similar in blocked and control groups. No significant difference was found between blocked and control groups in term of production of IFN-α and TNF-by CD8+ T cells.

Conclusion:

We concluded that blockade of PD-1 and Tim-3 is not an effective strategy to restore the function of CD8+ T-cells in CLL patients at the early stages of the disease. Further in vitro and in vivo studies are needed to more address the application of immune checkpoint blockade in CLL patients.

Keywords:

Chronic lymphocytic leukemia, PD-1, Tim-3, Immune checkpoint inhibitors