ABSTRACT

Cancer is the second most common cause of death in the world, and the number of people with the disease increases each year. The therapy of the disease currently stands on four pillars; surgery, chemotherapy, radiotherapy, and immunotherapy. Through the past few years, immunotherapy has become the fastest developing treatment modality. However, despite its unprecedented efficacy in some patients, the majority of patients still does not respond to the therapy. Therefore, there is a need to investigate the mechanisms that make immunotherapy inefficient. Cell-based cancer immunotherapy is the treatment modality which uses live *ex vivo*-produced tumor-targeting immune cells to treat cancer. One of the mechanisms that may compromise its therapeutic efficacy is the expression of inhibitory molecules on the surface of the produced immune cells. Tim-3 is the inhibitory molecule which attracts attention in recent years. Tim-3 expression in the tumor cells and the tumor-infiltrating immune cells is often associated with worse prognosis and more aggressive forms of the disease. However, its role in the *in vitro* or *ex vivo*-produced immune cells is difficult to predict. In this work, an *in vitro* study model which is based on *in vitro*-produced antigen-specific $CD8^+$ T cells with high expression of Tim-3 has been developed. Tim-3 expression in these cells was found to be conditioned by the presence of the cytokine IL-2 in the culture medium and the extent of its expression dependent on the cytokine concentration. Surprisingly, the expression of Tim-3 was substantially reversible because removal of the cytokine from the culture medium led to a substantial decrease of Tim-3 expression. Further analyses revealed that the extent of Tim-3 expression did not significantly affect the activation of the cells with their respective antigen. However, galectin-9, a Tim-3 ligand, showed a mild but significant inhibition of the antigen-mediated activation of $CD8^+$ T cells. The results of this work showed that the expression of Tim-3 in the *in vitro*-produced antigen-specific T cells is dynamic and that its engagement can partially inhibit antigen-mediated activation of $CD8^+$ T cells. These findings may have important ramification for designing the protocols for the production of immune cells for cancer immunotherapy.

Key words: Adoptive cell immunotherapy, immune resistance, dendritic cells, $CD8^+$ T cells, Tim-3, interleukin 2, galectin-9