Abstract

Adoptive T-cell therapy (ACT) with tumor infiltrating lymphocytes (TILs) is a promising therapy for patients with melanoma and other cancers. TILs involve culturing tumor fragments with IL-2 for 11-21 days, and re-infusing them into the patient following a rapid expansion protocol involving mitogenic anti-CD3 and irradiated PBMC. T-cells cultured from the tumor fragments are a heterogeneous mixture of cells representing CD4+, CD8+, and NK-cells with varying cytolytic activity and specificities. We developed a surrogate target cell line to evaluate the lytic potential of TILs in a Bioluminescent Redirected Lysis Assay (BRLA), enabling assessment of T-cell mediated killing in the absence of autologous tumor cells. Cytolytic activity can be assessed with and without engaging the T-cell receptor in 1-4 hours, assessing T-cell killing engaging the T-cell receptor and without, so-called lymphokine activated killer activities (LAK).

Methods

- Mouse mastocytoma P815 cells expressing the endogenous CD16 Fc receptor can bind anti-CD3 (OKT3), providing a potent TCR activation signal as a target cell line.
- The P815 Clone G6 was transduced with a lentiviral vector based on eGFP and Firefly Luciferase, sorted and cloned using the BD FACSAria II. Clone G6 was selected based on eGFP intensity analyzed using Intellicyte iQue Screener.
- Target cells and TILs of interest were cocultured +/- OKT3 to assess TCR activation (specific killing) or non-specific (lymphokine activated killing, LAK) respectively.
- Following 4hr of incubation, Luciferin was added to the wells and incubated for 5 min. Bioluminescence intensity was read using a luminometer. Percent cytotoxicity and survival were calculated using the following formula:

\[
\% \text{ Cytotoxicity} = 100 - \left( \frac{\text{Survival}}{100} \right) \\
\% \text{Survival} = \left( \frac{\text{Experimental \ survival}}{\text{Minimum \ survival}} \right) \times 100 \\
\text{Maximum \ signal} - \text{Minimum \ signal} 
\]

- Interferon gamma (IFN-γ) release in the media supernatant of cocultured TILs was analyzed by ELISA, and LAMP1 (CD107a, clone eBioH4A3) expression on TILs was analyzed on a flow cytometer to evaluate the cytotoxic potency of TILs.

Results

Figure 1. BRLA: cytotoxic efficiency of TILs correlates with IFN-γ and LAMP1

Figure 2. BRLA has comparable dynamic range compared with the standard chromium release assay

Figure 3. Direct correlation of cytotoxic efficiency of TILs with IFN-γ release

Figure 4. Direct correlation of Granzyme B and TNFs measures of M1053T and M1030T with cytolytic activity in BRLA

Conclusions

- The Bioluminescent Redirected Lysis Assay (BRLA) requires no radionuclides and is as efficient and sensitive as traditional cytotoxicity assays.
- Flow cytometric assessment of LAMP1 expression on TILs at individual time points demonstrates degranulation of cytotoxic T-cells relative to the potency shown by BRLA.
- The BRLA demonstrates similar to better potency than standard chromium release assay.
- BRLA also enables evaluation of the potency of TIL lytic activity. Comparison of BRLA with chromium release assay shows the efficiency and reliability of BRLA.
- BRLA has a linear relationship with IFN-γ release by TILs.
- Release assay of IFN-γ, TNFα, and Granzyme B by ELISpot is consistent with the cytotoxic efficiency of the TILs evaluated by BRLA.
- Future efforts will assess and contrast IFN-γ release and cytotoxicity assays as potency/release assays for TILs.

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