International Society for Cellular Therapy ISCT

Cytotherapy (2009) Vol. 11, No. 2, 206–217

informa healthcare

Modifying interleukin-2 concentrations during culture improves function of T cells for adoptive immunotherapy

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Background

Adoptive immunotherapy with cytotoxic T cells has shown promising clinical results in patients with metastatic melanoma and post-transplant-associated viral infections. Cell transfer therapies often require the ex vivo *expansion of large numbers of reactive lymphocytes. Therefore interleukin-2 (IL-2), a potent T-cell mitogenic cytokine that critically affects the features and effectiveness of T cells, is frequently added to cell culture media.*

Methods

We examined the influence of various IL-2 concentrations on cell growth, cytotoxicity, cytokine release and surface marker expression of tumor-infiltrating lymphocytes (TIL) during a standard 14-day rapid expansion phase. The study was conducted under good manufacturing practice (GMP) conditions, using approved reagents in a class 10 000 laboratory.

Results

T-cell cultures grown in very high IL-2 concentrations (600–6000 IU/mL) expanded massively and maximally secreted interferon (IFN)-γ in response to antigenic stimulation, but exhibited only low direct cytotoxicity. On the other hand, TIL cultures grown in low concentrations of IL-2 throughout the rapid expansion phase expanded to a lower extent and barely secreted IFN-γ but displayed high cytotoxic activity. A combined approach of starting with 10–120 IU/mL IL-2 during the first week, followed by increasing the IL-2 concentration to 6000 IU/mL during the second week, results in T cells that expand well, maximally produce IFN-γ and are highly cytotoxic against tumor cells.

Discussion

Fine tuning of the IL-2 concentration during ex vivo *expansion of T cells can yield high numbers of T cells with optimal features for clinical use.*

Keywords

adoptive immunotherapies, ex vivo *expansion, interleukin-2, T cell.*

Introduction

Adoptive cell transfer (ACT) immunotherapy engages the transfer of *ex vivo* activated and expanded immune effector cells. Immunotherapy with antigen (Ag)-specific T cells has the potential to be a powerful therapeutic modality for malignancies, in both adjuvant and metastatic settings, and provides an efficient prophylactic and therapeutic platform for post-transplant-associated viral infections [1]. Various methods have been developed to generate effector T cells from various sources, including peripheral blood, tumor-draining lymph nodes and tumor sites. Encouraging results have recently been demonstrated with an objective response rate of 51% by infusing autologous tumor-infiltrating lymphocytes (TIL) into immunodepleted patients with refractory metastatic melanoma [2]. In a later study, objective cancer regression was achieved by

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DOI: 10.1080/14653240802590391

employing genetically modified cytotoxic T cells derived from peripheral blood for ACT in advanced melanoma patients [3]. Chang *et al.* [4] executed a clinical study using T cells derived from tumor-draining lymph nodes for the treatment of metastatic renal cell carcinoma, achieving remission in 26% (9/34) of the patients. The transfer of T cells with specificity to viral Ag for therapeutic purposes has also provided remarkable results in the context of prophylaxis and treatment of virus-associated infections and diseases post-hematopoietic stem cell transplants (HSCT). Infusion of polyclonal cytomegalovirus (CMV)-specific cytotoxic T-cell (CTL) lines into recipients of allogeneic HSCT with persisting or recurring CMV infections showed a significant reduction of viral load in 71% (5/7) of the patients [5,6]. Prophylaxis and treatment of Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) was similarly successful [7]. Adoptive transfer of polyclonal EBV-specific T-cell lines protected 100% (60/60) of HSCT recipients against development of PTLD [8]; 83% (5/6) of patients receiving EBV-specific CTL after onset of lymphoma achieved complete remission [7].

Today, new sophisticated strategies for the generation of reactive T cells are under development. These strategies include genetic engineering of blood-derived T cells [9]. It has been suggested that the transduction of a gene encoding for T-cell receptor (TCR) directed against the mutated p53 molecule would render a wide range of epithelial tumors susceptible to ACT immunotherapy [10]. The production of T cells transfected with a plasmid DNA containing a human CD19-specfic chimeric Ag receptor gene further opens the possibility of treating hematologic malignancies [11], thus making this field even more attractive for the future.

The common feature of different types of ACT, regardless of the T-cell source, *ex vivo* manipulation and method of selection, is the expansion of effective T cells. A main component in the *ex vivo* expansion process is the mitogenic cytokine interleukin (IL)-2, which is commonly added during culture. Physiologically, IL-2 is produced by T cells following Ag presentation, which by themselves express the IL-2 receptor (R). The IL-2–IL-2R interaction stimulates the growth, differentiation and survival of Ag-selected T cells [12]. IL-2 is necessary for the development of T-cell immunologic memory [13] as well as induction of regulatory T-cell activity [14]. In addition, IL-2 facilitates the production of immunoglobulins by B cells [15] and induces the differentiation and proliferation of natural killer (NK) cells [16,17]. Because of its potent stimulatory capabilities for cytotoxic T cells and natural killer cells, recombinant IL-2 was approved by the Food and Drug Administration (FDA) for the treatment of metastatic renal cell carcinoma and melanoma in 1992 [18].

IL-2 is frequently used for the cultivation and expansion of T cells. However, IL-2 concentrations used for T-cell expansions in different ACT studies vary between 10 IU/mL and 6000 IU/mL [19–21]. Several studies have compared the effect of different IL-2 concentrations, but their only focus was the effect of IL-2 on proliferation rate [20,22]. Remarkably, the effects of various IL-2 concentrations on the critical functions of the cells obtained have never been investigated.

In 2003, Dudley *et al.* [21] published a new technology for the generation of autologous TIL from patients with metastatic melanoma. Tumor-specific TIL cultures were selected and underwent massive numerical expansion to generate high quantities of efficient T cells. These T cells were adoptively transferred back to the lymphodepleted patient and had the ability to mediate rejection of large, vascularized tumors. Using this technology, an objective response rate of 51% (18/35), including three complete responders, was achieved [2,23]. Noteworthy was that all patients had refractory disease and were heavily pre-treated with chemotherapy and/or immunotherapy, including high-dose IL-2. To date, such a high response rate has never been accomplished with any other modality. The Ella Institute at the Sheba Medical Center (Tel-Hashomer, Israel) adopted this promising therapy in 2005 [24].

The generation of anti-tumor-specific TIL consists of three major steps [21]: (1) isolation of TIL from metastases or primary tumor specimens; (2) growth of TIL cultures in IL-2-containing medium and selection of T lymphocytes with high specific activity against the tumor, by determining interferon (IFN)-γ levels after co-culture of TIL and melanoma cells; and (3) rapid, large-scale *ex vivo* expansion and activation of the selected melanoma-reactive T lymphocytes by using IL-2, anti-CD3 antibody (Ab) OKT3 and irradiated peripheral blood mononuclear cells from three allogeneic donors as feeder cells. Within 14 days, cultures expand to an average of 1392-fold (range 208–4271-fold) [24]. Starting a large-scale expansion with $30–50 \times 10^6$ T cells can lead to final cell numbers of up to