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Review

Cancer immunotherapy utilizing gene-modified T cells: From the bench to the clinic☆

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ABSTRACT

The immune system plays a critical role in the elimination and suppression of pathogens. Although the endogenous immune system is capable of immune surveillance resulting in the elimination of cancer cells, tumor cells have developed a variety of mechanisms to escape immune recognition often resulting in tumor outgrowth. The presence of immune infiltrate in tumors has been correlated with a good prognosis following treatment (Sato et al., 2005; Loi et al., 2013; Clemente et al., 1996; Galon et al., 2006). As such, immune cells such as T cells, have been harnessed in order to target cancer. Tumor reactive lymphocytes, called tumor-infiltrating lymphocytes (TILs) have been isolated and expanded from the tumor and reinfused back into patients for the treatment of melanoma. The promise of adoptive immunotherapy utilizing TILs as a robust treatment for cancer has been highlighted in patients with advanced melanoma with greater than 50% of patients responding to treatment (Dudley et al., 2005). Although TIL therapy has shown promising results in melanoma patients, it has proved difficult to translate this approach to other cancers, given that the numbers of TILs that can be isolated are generally low. To broaden this therapy for other cancers, T cells have been genetically modified to endow them with tumor reactivity using either a T cell receptor (TCR) (Parkhurst et al., 2009, 2011; Chinnasamy et al., 2011) or a chimeric antigen receptor (CAR) (Grupp et al., 2013; Park et al., 2007). This review will outline the origins and development of adoptive immunotherapy utilizing TILs leading to genetic modification strategies to redirect T cells to cancer. Potential hurdles and novel strategies will be discussed for realizing the full potential of adoptive immunotherapy becoming a standard of care treatment for cancer.

1. Introduction

The maintenance of a healthy host is largely dependent on the ability for the immune system to identify, suppress and eliminate pathogens. Although the endogenous immune system is rarely capable of eliminating established cancer, the presence of immune infiltrate in tumors has been correlated with good prognosis following therapy (Sato et al., 2005; Loi et al., 2013; Clemente et al., 1996; Galon et al., 2006). Therefore, components of the immune system such as T cells have been utilized in order to target cancer. This has involved isolating and expanding tumor reactive lymphocytes from the tumor, called tumor-infiltrating lymphocytes (TILs). The promise of adoptive immunotherapy utilizing TILs as a robust treatment for cancer has been recently highlighted in patients with advanced melanoma, with greater than 50% of patients responding to treatment (Dudley et al., 2005). However, TILs are challenging to isolate from cancers other than melanoma. To overcome this problem, T cells have been genetically engineered endowing them with tumor reactivity. This has involved modifying T cells with αβ T cell receptor (TCR) transgenes (Parkhurst et al., 2009, 2011; Chinnasamy et al., 2011). TCR modified T cells have had some promising results in clinical trials in melanoma patients (Morgan et al., 2006). However, tumors are capable of escaping detection by the immune system by downregulating molecules

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such as MHCI. Therefore, recombinant receptors called chimeric antigen receptor (CAR) (Grupp et al., 2013; Park et al., 2007) have been developed, arming the T cell with anti-tumor activity regardless of MHCI expression. The tremendous promises of the approach have been recently highlighted utilizing CAR T cells specific for CD19, which has resulted in remarkable responses in hematological malignancies (Maude et al., 2014). In this review, we outline the origins and development of adoptive immunotherapy utilizing TILs, TCR modified T cells and CAR modified T cells. Finally we discuss potential hurdles and novel strategies for the potential of adoptive immunotherapy becoming a standard of care treatment for cancer.

2. Adoptive immunotherapy

2.1. Early preclinical studies and clinical trials

It has long been proposed that the presence of a lymphocytic infiltration within tumors is associated with a good prognosis. In 1972, it was reported that a gastric cancer patient with hepatic metastases had a total regression of metastases in his liver in the absence of therapy (Rosenberg et al., 1972). The dense infiltration of lymphocytes in the resected gastric biopsy suggested the importance of these TILs against the growing cancer (Rosenberg et al., 1972). The presence of TILs have since been associated with a favorable prognosis in other cancers including ovarian (Sato et al., 2005), breast (Loi et al., 2013), melanoma (Clemente et al., 1996) and colon cancer (Galon et al., 2006)

Whilst TILs are capable of recognizing tumor associated antigens through their endogenous TCRs, the small number of TILs isolated from patient tumors without expansion are insufficient for clinical intervention. The discovery of interleukin-2 (IL-2) (Smith et al., 1980a, 1980b) allowed for the *ex vivo* expansion of TILs isolated from patients, with early clinical trials initiated by the pioneers of adoptive immunotherapy, Rosenberg et al. (Rosenberg et al., 1988a; Topalian et al., 1988; Lotze and Rosenberg, 1986) at the National Institutes of Health. The first clinical trial of adoptive immunotherapy involved the administration of IL-2 expanded TILs from 12 patients with advanced renal or breast cancer, colon carcinoma or melanoma. A single dose of cyclophosphamide was used to precondition the patients who were infused with 8×10^9–2.3×10^{11} cells and varying doses of IL-2. Partial tumor regression was reported in one patient with renal carcinoma, one patient with breast cancer and one patient with melanoma (Topalian et al., 1988). These encouraging results led to further trials to assess the combination of lymphodepleting regimens and the use of IL-2 for enhancing the efficacy of adoptive T cell therapy.

Early preclinical and clinical studies for metastatic melanoma were pioneered by Rosenberg et al. (Rosenberg et al., 1988a; Lotze and Rosenberg, 1986). Tumor reactive TILs were cultured with fresh melanoma samples or melanoma cell lines, and assessed for their ability to secrete cytokines and lyse target cells *in vitro* (Topalian et al., 1987). Following selection for antigen recognition, these TILs were then activated and expanded *ex vivo* in the absence of an immunosuppressive tumor microenvironment. Patients also received high doses of IL-2 to support the activation and proliferation of infused T cells (Forni et al., 1986; Testa et al., 1990). In a preliminary clinical trial with metastatic melanoma, Rosenberg et al. found that 11/20 of patients treated with this regimen had objective regression of their tumors in various sites of the body that lasted from 2 to greater than 13 months (Rosenberg et al., 1988a). The protocol used in this trial became the foundation of future clinical trials.

In these early trials, although some patients experienced long lasting regression, the majority of anti-tumor responses were short-lived. To determine the persistence and distribution of the infused TILs in patients, Rosenberg et al. retrovirally transduced TILs to express the neomycin resistance gene, which allowed the cells to be tracked following infusion (Rosenberg et al., 1990). Using polymerase chain reaction (PCR) analysis, they reported minimal numbers of TILs in the patients' peripheral blood and tumor biopsy and that these numbers decreased significantly after 3 weeks. The relatively short life span and poor infiltration of the infused TILs into the tumor bed warranted further investigation to enhance the tumoricidal capacity of the transferred T cells.

2.2. Lymphodepletion in adoptive immunotherapy

Murine models have demonstrated that lymphodepletion using nonmyeloablative sublethal total body irradiation (TBI) prior to TIL infusion greatly augmented the efficacy of adoptive immunotherapy (Wrzesinski et al., 2010; Gattinoni et al., 2005a). The mechanisms as to why lymphodepletion is beneficial prior to the infusion of T cells are still not completely understood. It has been shown that there is an increase in freely available homeostatic cytokines such as IL-2, IL-7 and IL-15 in the absence of cellular 'sinks', a phenomenon where endogenous lymphocytes such as T cells and natural killer cells (NKs) compete with transferred T cells for cytokine support (Wrzesinski et al., 2010; Gattinoni et al., 2005a; Klebanoff et al., 2005a). Elimination of immunosuppressive cells such as myeloid derived suppressor cells (MDSCs) and T regulatory cells (T_{regs}) and their secreted inhibitory cytokines such as IL-10 may also play a role in this enhanced anti-tumor effect (Gajewski et al., 2013; Gabrilovich et al., 2012). It is also believed that damage to the mucosal surfaces releases toll like receptor (TLR) agonists, inducing dendritic cells (DCs) to mature and stimulate expansion of the activated infused T cells (Kieper et al., 2005; Hill et al., 1997). Therefore, it appears that lymphodepletion using irradiation and chemotherapy are able to skew the tumor microenvironment, depleting pro-tumoral elements and subsequently allowing for a beneficial shift in the ratio of infused T cells to suppressive cells.

These preclinical findings prompted the investigation of the preconditioning regimen including TBI and lymphodepleting prior to adoptive transfer, which results in the temporary elimination of host immune cells from the patient. Several clinical trials treating patients with metastatic melanoma utilized increasing doses of lymphodepletion, achieving objective response rates of 49–72% (Dudley et al., 2005; Rosenberg et al., 2008, 2011) (the response evaluation criteria in solid tumors (RECIST) was used in these studies). In a report consisting of three sequential clinical trials of 93 patients with measurable metastatic melanoma, the combination of chemotherapy, TBI and adoptive immunotherapy led to the regression of melanoma metastasis in the lung, liver, brain, bone, lymph nodes and subcutaneous tissue, which resulted in objective response rates of 72%, with some complete responses reported for more than 3 years at the time of publication (Rosenberg et al., 2011). The results were more remarkable given that some tumors were rendered inoperable due to their size and location.

2.3. Optimal phenotype of infused T cells

While the *ex vivo* culture conditions for T cell expansion favor a more effector phenotype, several studies have focused on investigating which phenotype of infused T cells will thrive and induce the greatest anti-tumor effect following infusion. Early pre-clinical and clinical protocols for the generation of TILs involved the activation and expansion of T cells into large numbers *in vitro* with IL-2. T cells with attributes such as interferon-γ (IFN-γ) secretion (Barth et al., 1991) and cytotoxicity toward tumor cells (Aebersold et al., 1991; Schwartzentruber et al., 1994) were isolated and infused into patients. These T cells were highly activated and had an effector

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