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Engineered Drug Resistant Cell-Mediated Immunotherapy

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1. Introduction

In principle, gene therapy is the introduction of a nucleic acid sequence(s) into target cells to prevent or cure a wide range of diseases. Such a therapeutic approach was initially directed toward the correction of inherited disorders. For example, one strategy involves introduction of a corrected gene into cells followed by transplantation of the genetically modified cells into patients. But technological hurdles such as inadequate transfer of genetic materials into cells, thus leading to low transgene integration, and safety concerns with gene transfer methodologies have limited the use of gene therapy. However, significant improvements in gene delivery systems that are both efficient and safe have now directed the growth of gene therapy, as evidenced by several hundred gene therapy-based clinical trials being conducted worldwide. Advancements in all areas of gene therapy now allow for the development of genetically engineered immunocompetent cell-based therapies for cancer.

The therapeutic role of immune cells to control malignancies has been well established. For example, immunocompetent hosts frequently reject transplanted tumors while tumors can easily be established in immunosuppressant hosts. In vitro studies have demonstrated that immunocompetent cells exhibit powerful cytotoxicity toward a broad range of cancer cells and tumorigenic animal models have shown that these cells can infiltrate tumors resulting in tumor regression. Tumor infiltration by immunocompetent cells indicates favorable prognosis in various cancers, such as melanoma, colon, ovarian cancer, basal cell carcinoma, and lung cancer (Haanen et al., 2006; Halliday et al., 1995; Inoue et al., 2000; Kerr et al., 1998; Pages et al., 2005; Zhang et al., 2003). These observations have led to the development of cell based immunotherapy strategies to target cancer.

However, intensive chemotherapy regimens, the frontline therapy to treat patients with advanced cancer, frequently lead to non-specific cellular toxicity to adoptively transferred immunocompetent cells and to hematopoietic stem cells. One strategy to combat drug-induced toxicity is to genetically engineer immune cells to make them drug resistant. Several genes, such as methylguanine methyltransferase (MGMT), dihydrofolate reductase (DHFR), cytidine deaminase (CD), and multidrug resistant protein (MDR-1) have been identified that can confer drug resistance to anti-cancer immune cells, and advances in gene therapy techniques have made it possible to test the feasibility of using the cDNAs encoding these sequences in drug resistant gene therapy studies. The ability to generate chemo-resistant immune cells can be exploited to test novel anti-cancer therapies, such as "Drug

Resistant Immunotherapy" (or DRI) whereby drug resistant immunocompetent cells can be administered in conjunction with chemotherapy. Such a treatment modality can significantly enhance the generation of anti-tumor immunity that is quantitatively and qualitatively superior to that achieved by either cellular immunotherapy or chemotherapy alone. DRI can also benefit from the ability to selectively expanding the modified cells in vivo by administering specific chemotherapeutic agents thereby mitigating the problem of inefficient gene transfer to these cells. The anti-cancer effectiveness of DRI based therapy has been successfully demonstrated using either drug resistant bone marrow or immunocompetent cells with intrinsic cytotoxic capabilities. The therapeutic benefits afforded by a DRI based strategy can potentially be enhanced by the synergistic effects between chemotherapy and immunotherapy. For example, chemotherapy mediated lymphodepletion prior to adoptive transfer of drug resistant immunocompetent cells can mitigate immunosuppressive mechanisms that are advantageously exploited by progressive tumor cells to evade immune recognition. With the recent advances in *ex vivo* gene transfer technology, drug resistant variants of i) tumor specific lymphocytes, and their genetically engineered counterparts such as those harboring transgenic $\alpha\beta$ T cell receptor (TCR)/cancer antigen receptor (CAR) modifications, and ii) lymphocytes with non-specific cytotoxic potentials, can be bioengineered to treat various malignancies.

However such a gene therapy based approach can be limited by inadequate transfer of cDNA that encodes drug resistant genes. The ability to genetically engineer immune cells, specifically hematopoietic cells, has greatly advanced in recent years. It is generally accepted that recombinant retroviral vectors, specifically those based on HIV-1) are the most efficient gene transfer systems for the *ex vivo* modification of hematopoietic cells. Retroviral systems have the advantage that i) recombinant viruses are now relatively easy to generate and characterize, ii) most components for retroviral transfer systems are commercially available, iii) retroviral gene transfer results in stable integration of the transferred nucleic acid sequence into the genome of the target cell, and iv) depending on the target cells, the efficiency of gene transfer can approach 100%.

This chapter will discuss cancer immunotherapy with genetically engineered immune cells and the feasibility of employing drug resistant variants of these cells during chemotherapy that can potentially augment such a cell therapy based approach.

2. Combining drug resistant immune cell therapy with chemotherapy: A new paradigm in cancer treatment

Despite relentless efforts worldwide to improve upon conventional treatment modalities for cancer, chemotherapy remains a much needed frontline therapy. Several cytotoxic agents, such as anti-metabolites, alkylating agents, anthracyclines, DNA methyltransferase inhibitors, platinum compounds and spindle poisons have been developed to kill cancer cells. However, they are not uniformly effective, and the introduction of these agents with novel state-of-the-art therapies, such as immunotherapies, is problematic. For example, these agents can be detrimental to the establishment of robust anti-tumor immunocompetent cells due to the non-specific cellular toxicity of many anti-cancer agents. Intensive chemotherapy frequently administered to treat patients with advanced cancer can result in lymphopenia, which decrease the numbers and function of potential anti-cancer T cells in the blood thereby blunting the anti-tumor immune responses (Liseth et al., 2010). Tumor drug resistance can also develop, resulting in ineffective chemotherapy treatment

(Michael & Doherty, 2005). In addition, induced secondary cancers and long-term survivorship issues limit the effectiveness of some cytotoxic chemotherapy agents (Perry et al., 1998). If the chemotherapy regimens that are transiently effective can be combined with immunocompetent cell therapies, then it is predicted that a significant improvement of anti-neoplastic therapy can be achieved. However, because chemotherapy regimens are toxic to immunocompetent cells, the co-administration of these treatments reduces or eliminates the effectiveness of the immunocompetent cells.

One strategy to combat drug-induced toxicity is to genetically engineer immunocompetent cells to make them drug resistant. This strategy facilitated the development of a DRI-based anti-cancer technology that combines the therapeutic effectiveness afforded by drug resistant immunocompetent cells with conventional chemotherapy. This strategy involves 1) the use of lentivirus mediated applications to introduce cDNAs that encode for drug resistant genes into immunocompetent cells, and 2) administration of the genetically-modified immunocompetent cells in conjunction with chemotherapy to enhance tumor cell clearance. The novel features of such a strategy are 1) it can be more potent in tumor elimination than the individual administration of chemotherapy or immunotherapy, 2) it can easily be integrated with other conventional treatment modalities, such as surgery and antibody/vaccine based immunotherapy, 3) it is applicable to patients with any disease stages, and, 4) less aggressive chemotherapy can be applied during DRI therapy applications, thereby reducing the development of tumor drug resistance and induction of secondary cancers. Several strategies have been identified that can be used to confer drug resistance to targeted cells, and advances in gene therapy techniques have made it possible to test the feasibility of using the cDNA encoding proteins that confer resistance in drug resistance gene therapy studies. A number of proteins have been identified that confer drug resistance, but this chapter will focus on four that have already been used to confer resistance specifically to hematopoietic cells.

2.1 Drug resistant genes

DHFR regulates folate homeostasis by controlling the synthesis of purines and pyrimidines. Anti-folate drugs such methotrexate and trimetrexate, are inhibitors of DHFR. Gene therapy strategies have exploited the use of DHFR mutants that can confer resistance to antifolates. For example, bioengineered hematopoietic stem cells (HSCs) harboring a mutated DHFR transgene protects mice from an antifolate dose that is lethal to non-modified HSCs. (Allay et al., 1997, 1998a; Spencer et al., 1996.). However, HSCs bypass drug induced toxicity by increased nucleoside transport. To circumvent such effects, nucleoside transport inhibitors have been co-administered with antifolate drugs to significantly increase the population of cells modified with DHFR mutants, specifically L22Y (Allay et al., 1998b; Warlick et al., 2002).

The therapeutic efficiency of a single drug resistant based gene therapy can be significantly enhanced by genetically engineering dual drug resistant anti-cancer cells. Such a strategy can be particularly effective in treating tumors that respond to drug combinations that exhibit synergistic (or additive) effects. The combination of methotrexate and a cytosine nucleotide analog, cytosine arabinoside (Ara-C) has been successfully used in patients with Non-Hodgkins lymphoma (Fisher et al., 1993; Khouri et al., 1998). Ara-C is inactivated by CD which is involved in the salvage of pyrimidine compounds and in pyrimidine metabolism. The feasibility of bioengineering cells resistant to both an antifolate and Ara-C have led to the development of gene therapy strategies based on the generation of dual

drug resistant cells (Sauerbrey et al., 1999). Transplantation of dual drug resistant bone marrow cells generated by the retroviral transfer of a fusion construct: mutDHFR-CD encoding for mutant DHFR and CD genes, into myoablated mice significantly reduced the growth of established leukemia, while protecting hematopoietic cells (Budak-Alpdogan et al., 2004).

During cancer progression, the tumor acquires resistance to multiple natural products by the expression of the MDR-1 gene. This gene encodes a membrane glycoprotein, known as P-glycoprotein (P-GP) involved in the transport of metabolic byproducts across the cell membrane. The P-GP protein displays broad specificity towards several structurally unrelated chemotherapy agents. Thus, pleiotropic drug resistance can be conferred to cells by the transfer of nucleic acid sequence that encodes for MDR-1. MDR-1 gene was one of the first candidate genes to be exploited in the context of drug resistant gene therapy to confer protection to bone marrow cells. (Abonour et al., 2000; Bunting et al, 2000; Cowan et al, 1999; Moscow et al., 1999; Sellers et al., 2001; Sorrentino et al, 1992). These initial studies, and others, led to the development of the field of drug resistance gene therapy for cancer. One strategy is to harvest HSCs from cancer patients and genetically modify them to express the MDR-1 gene followed by transplantation into patients. During engraftment, chemotherapy is administered to selectively enrich for modified cells *in vivo*, which leads to lower cytotoxicity upon repeated chemotherapy treatments.

Among the drug resistant genes studied, MGMT is among the most promising. This gene encodes for human alkyl guanine transferase (hAGT), a DNA repair protein that confers resistance to the cytotoxic effects of alkylating agents, such as BCNU and temozolomide (Davis et al., 1997; Liu et al., 2002; Maze et al., 1996). Tumor cells have been shown to express high levels of AGT, which can be an effective mechanism of tumor cell drug resistance. To circumvent AGT-mediated resistance, alkylating agents have been administered in combination with inhibitors of AGT, namely 6-Benzyl Guanine (6-BG) (Dolan et al., 1989). Although 6-BG sensitizes tumor cells to alkylating agents, drug induced toxicities such as myelosuppression severely limit the use of these combined agents. To overcome this limitation, several BG-resistant variants of AGT have been generated and used in gene transfer studies. Among them, the P140KMGMT variant has been well characterized with respect to drug resistant gene therapy. (Gerull et al., 2007; Larochelle et al., 2009; Neff, et al., 2005; Pollok et al., 2003; Sawai et al., 2001; Zielske et al., 2003).

2.2 Evaluation of drug resistant immunotherapy (DRI)

The development of drug resistant genes along with the advancements in gene delivery systems has allowed for the chemoprotection of immunocompetent cells. Genetic engineering of drug resistant hematopoietic cells has been well documented (Allay et al, 1998a, 1998b; Davis et al, 1997; Maze et al, 1996; Zhao et al, 2008). Transplantation of modified HSCs has been shown to protect the hematopoietic system from chemotherapy-induced toxicity, and this strategy has been used to enrich the percentage of circulating gene-modified cells. The advantages to the establishment of such chemo-resistant bone marrow cells are two-fold. First, chemotherapy can be administered, possibly at a higher frequency and at higher doses. For example, transplantation of the DHFR mutant L22Y-modified HSCs allowed for the administration of the antifolate drug, trimetrexate (TMTX) at concentrations that are lethal to animals not receiving the genetically altered bone marrow cells (Allay et al., 1997, 1998; May et al., 1995; Spencer et al., 1996). Secondly, tumor targeting T lymphocytes can be expanded during chemotherapy challenges, leading to increased tumor infiltration and potentially increased tumor clearance

The anti-cancer effectiveness of combining drug resistant HSCs, immune-modulating agents, and chemotherapy was evaluated by combining the administration of an antifolate based chemotherapy, trimetrexate (TMTX), along with anti-CD137-based immunotherapy in mice transplanted with anti-folate resistant HSCs (McMillin et al., 2006). Mice were initially transplanted with L22Y-DHFR-modified bone marrow and were allowed to reconstitute with drug resistant hematopoietic cells. The reconstituted mice were implanted with sarcoma cells, i.e. the AG104 sarcoma cell line. The tumor bearing animals were exposed to treatments comprising of either anti-CD137, TMTX, or the combination of anti-CD137 and TMTX. Chemotherapy alone mediated tumor regressions only during the treatment phase. However, once this treatment ended all animals in this treatment group experienced rapid growth of their tumor. Similarly, administration of immunotherapy alone regressed tumors in the majority of animals, but only 40% of the animals achieve long-term tumor clearance. However, all animals treated with chemotherapy along with immunotherapy resulted in complete tumor regressions. Such an observation confirmed the existence of possible synergism between immunotherapy and chemotherapy in the context of a drug resistant immunotherapy strategy. Importantly, application of the DRI based strategy by combining chemo-and immunotherapies can lead to the induction of immunological memory. Such an effect was demonstrated by infusing splenocytes isolated from mice in the combined treatment group into untreated tumor bearing animals. The adoptive transfer of potential immunocompetent cell populations led to reductions in tumor burdens and extension of survival of the recipient mice. These studies have important implications in designing treatment modalities to generate a robust antitumor response that may lead to eradicate residual tumor. Such a strategy will involve the co-administration of chemo-protected immunocompetent cells with chemotherapy that mediate a fast antitumor response to reduce tumor burden followed by the induction of immune memory cells that are sustained over a long time to eliminate any persistent cancer cells.

Although initial studies focused on immunotherapy driven by HSCs, which is the source of all immune cells, other proof-of-concept studies were directed toward the evaluation of specific immunocompetent cells as mediators of DRI. For example, one study exploited the use of genetically engineered drug resistant variants of the anti-cancer immune cells, NK92 and T-ALL104 cells, in combination with temozolomide (Dasgupta, et al., 2010). These cells were selected based on their direct immunotherapeutic properties to mediate robust anti-tumor properties without the requirement of MHC presentation (Gong et al., 1994; Tam et al., 1999; Tonn et al., 2001). TALL-104 cells represent a leukemic T cell line that has surface markers typical of both cytotoxic T lymphocytes and natural killer cells and adoptive immunotherapy with TALL-104 cells has induced long-term complete or partial remissions in tumor bearing animals (Cesano et al., 1998; Georger et al., 2000). In this study, a SIV-based lentiviral system was employed to deliver the drug resistant variant P140KMGMT into the immunocompetent cell lines NK-92 and TALL-104, and in the myelogenous leukemia cell line, K562, which is a target for both NK-92 and TALL-104 cells. Using in vitro survival and cytotoxicity assays it was demonstrated that 1) the genetically-modified cells developed significant resistance to the alkylating drug temozolomide when compared to the untransfected wild type cells, 2) genetic modification of the immune effector cells did not alter their ability to kill target cells, and 3) genetically altered cells were active in killing target cells after drug treatments, while the killing effectiveness of the unmodified effector cells is significantly diminished after a chemotherapy challenge. However, genetically modified drug resistant cells killed virtually all of un-modified K562 target cells in the

presence of drug, which was significantly higher compared to the killing effectiveness of the non-modified effector cells.

These *in vivo* and *in vitro* proof-of-concept studies demonstrate that drug resistant immunocompetent effector cells are superior cytotoxic effectors during a chemotherapy challenge. This is a significant finding which can potentially be combined with current cell-based and adoptive immunotherapies. Regression of large, vascularized tumors has been shown in patients with refractory metastatic melanoma. However, for maximum effectiveness a lympho-depleting regimen is necessary prior to autologous lymphocyte cell transfer (Rosenberg & Dudley, 2004). Generation and expansion of drug-resistant lymphocytes *ex vivo* can allow, in this setting, for the administration of immunocompetent cell-based therapy concurrently with chemotherapy, potentially improving tumor clearance while anti-tumor immunity is established and maintained. In this scenario, non-transduced lymphocytes can continually be depleted using a selective chemotherapy treatment, which could be repeatedly applied during the administration of adoptive immunotherapy. The co-administration of chemo- and immunotherapies could then lead to long-term tumor clearance. Thus, it is anticipated that the T lymphocytes with memory phenotypes are suitable candidates to be incorporated into future DRI studies to target advanced cancer.

3. Synergism between chemotherapy and immunotherapy can benefit drug resistant immunotherapy

Combining chemotherapy with immunotherapy is an attractive strategy to enhance the effectiveness of both treatments, but initial combination strategies consisting of high-dose chemotherapy combined with interleukin-2 (IL-2) were no better than chemotherapy alone (Pollera et al., 1994; Rinehart et al., 1992). However, studies in the last decade have demonstrated potential synergistic effects between chemotherapy and immunotherapy (Fridlender et al., 2010; Lake et al., 2005; Ramakrishnan et al., 2011). Conventional chemotherapy can augment immunotherapy in several ways: 1) induction of chemotherapy mediated-lymphodepletion leading to i) enhanced persistence of the tumor reactive T lymphocytes, ii) increase in tumor trafficking by the tumor responsive T cells (Dudley et al., 2002a, 2005), iii) modulation of immunosuppressive factors (Cui et al., 2009), and iv) promotion of differentiation of central memory effector cells (to augment vaccine based strategies) (Badovinac et al., 2005; Wrzesinski et al., 2007), and 2) chemotherapy induced sensitization of tumor to the immunocompetent cells by i) the induction of stress responsive molecules on tumor surface and ii) increasing the availability of tumor antigens to “boost” the T cell response (Fridlender et al., 2010). Thus a DRI therapy approach that integrates both chemo- and drug resistant immune cell therapies can significantly benefit from such partnerships.

3.1 Chemotherapy induced lymphopenia

Several clinical trials with melanoma patients support the concept that adoptive transfer of genetically engineered T lymphocytes may not be sufficient to improve treatment outcomes without lymphodepletion. For example, in the treatment of patients with advanced melanoma, anti-tumor response rate was observed only after adoptive transfer of T lymphocytes (with or without engineered specificity towards melanoma antigens) with lymphodepleting regimens (Dudley, et al., 2001, 2002b, 2005; Hughes et al, 2005). Lymphodepletion is thought to provide space in the lymphoid compartment thereby

allowing robust establishment of the transferred lymphocytes (Klebanoff et al., 2005) which resulted in the induction of faster and more efficient immune response with enhanced anti-tumor properties (Dudley et al., 2002; Rosenberg & Dudley, 2004; Wang et al., 2005a, 2005b). Lymphopenia also induces the rescue of memory T cells as shown in the treatment of mice with established melanoma. For example, the anti-tumor efficacy of an immunotherapy comprising an oncolytic vaccinia virus expressing CD137 T-cell costimulatory molecule is significantly enhanced when animals were lymphodepleted prior to vaccination (Kim et al., 2009). In addition, T regulatory cells (Tregs) have been implicated as having potent immune suppressive functions, and clinical trials have indicated that depleting or inhibiting such cells can increase anticancer efficacy (Phan et al., 2003). The number of Tregs can be reduced by chemotherapy and also by a combination of an adenoviral based immunogene therapy (Fridlender et al., 2010).

3.2 Chemotherapy induced upregulation of stress antigens on tumor surface

Chemotherapy can sensitize tumors to augment immunotherapy by the up-regulation of tumor specific antigens that are recognized by the activating receptors expressed by NK and $\gamma\delta$ T cells, thereby leading to an increase in tumor clearance (Nausch & Cerwenka, 2008). It has been reported that various cancer cells exposed to drugs upregulate stress-associated molecules MIC-A, MIC-B, and UL-16 binding proteins which are recognized by immunocompetent NK and $\gamma\delta$ T cells through their MHC-independent NKG2D/TCR pathways. Such innate HLA-independent interactions lead to the activation of anti-tumor properties of these cells, as has been demonstrated by $\gamma\delta$ T cell mediated destruction of glioblastoma cells exposed to temozolomide, the frontline chemotherapy agent in the treatment of patients afflicted with glioblastoma multiforme (GBM) (Lamb, 2009). This mode of immune cell-activation opens the possibility of testing a treatment modality that can combine immunotherapy based on drug resistant variants of NK or $\gamma\delta$ T cells and chemotherapy to target cancer types that express these activators during drug treatments. It was also demonstrated that in mouse models of colon and mammary cancer, applications of chemotherapeutic drugs upregulated the expression of a tumor cell surface receptor, mannose 6-phosphate receptor (Motyka et al, 2000). This receptor is implicated in the uptake of granzyme B released by CTLs upon contact with tumor cells, thereby establishing a synergy between chemotherapy and immunotherapy (Ramakrishnan et al, 2010).

3.3 Enhancement of tumor antigen presentation

Chemotherapy induced tumor cell apoptosis can liberate massive amounts of tumor specific antigens that are duly processed by antigen presenting cells and the processed antigens are presented, in association with MHC class I molecules, to CTLs leading to an increase in antigen presentation (Lake et al, 2005). Thus chemotherapy can augment immunotherapy by increasing antigen presentation, which can 1) lead to T lymphocyte expansion and increased lymphocyte infiltration of solid tumors and 2) mediate cancer vaccination effects. For example, it has been shown that chemotherapy can prime the host's immunity and enhance antitumor responses (Nowak et al., 2003a). Antitumor cytotoxic T lymphocytes (CTLs) showed increased proliferation because of increased tumor apoptosis when chemotherapy was administered, and incorporating immunotherapy with chemotherapy extended animal survival (Nowak et al., 2003b). Importantly, this study showed that the delivery of chemotherapy before immunotherapy is more effective than after immunotherapy (Nowak et al., 2003a).

4. Potential immunocompetent cell sources for implementing DRI

Our knowledge about the host immune response to cancer has led to the development of immunocompetent cell-based therapeutics. Two distinct classes of cells that are defined by their mechanism to invoke anti-tumor immunity have been exploited: i) the widely used tumor specific T lymphocytes, which must be primed prior to tumor cell killing, and ii) non-specific MHC-unrestricted effector cells with intrinsic tumor killing properties.

4.1 Tumor-directed T lymphocytes

T cells mediate their potent anti-tumor effectiveness by their ability to recognize a wide spectrum of antigens expressed on the tumor surface. These tumor associated antigens are processed by the antigen presenting cells, such as dendritic cells into smaller peptides which are presented to T cells in combination with MHC complexes. T cell activation occurs after the recognition of the peptide-MHC complex via their antigen specific receptor, i.e. TCR-CD3 complex. The T cell receptor is a heterodimer composed of either α and β or γ and δ polypeptide chains. Each chain of the TCR is composed of a variable region (V) and a constant region (C). The V region determines the antigen binding specificity of the TCR. The vast majority of peripheral blood T lymphocytes and TCR⁺ thymocytes have $\alpha\beta$ TCR while epithelial T cells contain the $\gamma\delta$ TCR. The TCR heterodimer is associated with the CD3 complex. The CD3 complex is necessary for i) the expression of TCR on the T cell surface and ii) activation of T cells by signal transduction when the TCR binds to its specific polypeptide primed MHC complex. Following the recognition of peptide-MHC, the CD3 complex initiates signal transduction pathways to mediate cell proliferation, cytokine secretion and activation of T cell anti-tumor properties (Chan et al.; 1992; Punt et al., 1994). Several tumor antigens such as melanoma/melanocyte differentiation antigens (MART-1 and gp100) and NY-ESO-1 cancer-testis antigen have been identified (Cormier et al., 1998; Morgan, et al., 2003; Zhao et al., 2005). The tumor antigens can activate a large number of T lymphocytes that can infiltrate the tumor (TIL). These immune cells have been widely employed during the administration of ACT to treat cancer. ACT involves i) the isolation of tumor infiltrating lymphocytes, either from a surgically removed tumor or from the peripheral blood, ii) ex vivo expansion of the selected cells in the presence of cytokines, and iii) infusion of the expanded cells back into the patient, typically after 'conditioning' of the patient with lymphodepleting regimens comprised of either chemotherapy or total body irradiation (TBI). It is now well established that ACT can establish or augment immunity and eradicate malignant cells.

Following the discovery of a large number of tumor antigens, TILs directed against such antigens have been successfully generated, mainly from melanoma but also from renal cell carcinoma and glioma (Dillman et al., 1991; Figlin et al., 1997; Kradin et al., 1989; Quattrocchi et al., 1999). TILs mediated tumor regression when transferred into tumor-bearing mice. ACT with TILs directed against melanoma antigens have proven successful since patients with melanoma are immunized against antigens expressed by their own tumors and melanoma tumors generate relatively higher quantities of melanoma antigen specific T lymphocytes. However, the use of TILs in the treatment of patients with cancer other than melanoma has met with limited success, possibly due to the presence of low number of cytotoxic T lymphocytes within TILs (Finke et al., 1994; Hom et al., 1993; Schwartzentruber et al., 1992). Therefore, improvements in the usefulness of TILs are needed, and DRI studies are in progress to determine if such modifications can improve the therapeutic potential of these cells.

4.1.1 Chimeric $\alpha\beta$ TCR modified T cells

In general, $\alpha\beta$ T lymphocytes display low affinity TCRs. Therefore, success with ACT with tumor directing $\alpha\beta$ T lymphocytes is limited by the difficulty in isolating high affinity $\alpha\beta$ T lymphocytes that exist in low numbers in vivo and in ex vivo expansion of these cells to generate adequate quantities for in vivo anti-tumor efficacy. Furthermore, tumor derived immunosuppression mechanisms reduce the number of tumor specific $\alpha\beta$ T cells in circulation. As a means of enhancing the anti-cancer efficacy of $\alpha\beta$ T lymphocytes, these cells can be genetically modified to express transgenic α and β TCR chains, which can be derived from T cell clones specific for tumor-associated antigens. These genetically engineered CTLs harboring a transgenic $\alpha\beta$ TCR, in addition to their native $\alpha\beta$ TCR, acquire the same antigen specificity as the high affinity T cells from which the TCR was cloned. Several CTLs harboring transgenic TCRs have now been developed that are directed specifically to tumor antigens, such as MART-1, gp100, NY-ESO-1 and CEA, resulting in tumor elimination in animal models (Abad, et al., 2008; Morgan, et al., 2003; 2006; Kessels et al., 2001; Stanislawski et al., 2001; Wargo et al., 2009; Xue et al., 2005). Bicistronic viral vectors encoding cDNA sequences for both α and β chains have been successfully incorporated into retroviral based strategies to transfer $\alpha\beta$ transgenic TCR into T lymphocytes (Yang et al., 2008). The functional efficacies of the engineered CTLs have also been improved by codon optimizing α and β sequences that result in increased surface expression of the transgenic TCRs (Jorritsma et al., 2007; Scholten et al., 2006). The chimeric $\alpha\beta$ T cells can be rapidly expanded ex vivo to produce sufficient quantities of tumor reactive cells and after adoptive transfer to patients display potent MHC-restricted cytotoxic activity against tumor cells expressing the specific epitope. ACT with tumor directed chimeric $\alpha\beta$ T lymphocytes have been widely used to treat various malignancies, particularly melanoma, because of the ease of isolating and expanding melanoma reactive CTLs ex vivo.

However, the genetic engineering of CTLs that express the transgenic $\alpha\beta$ TCR along with their endogenous TCR has inherent disadvantages. The endogenous TCR can compete with the transgenic $\alpha\beta$ TCR to bind to the initiator CD3 molecule. Consequently, the chimeric $\alpha\beta$ TCR-modified CTLs may suffer from reduced activation leading to a decrease in affinity of the transgenic $\alpha\beta$ TCR towards specific tumor antigens. Furthermore rearrangements between the chimeric and the naïve TCR chains can induce new and unwanted reactivities. To combat such undesired consequences TCR negative lymphocytes have been modified to harbor $\alpha\beta$ transgenic TCR while the sequences of such chimeric $\alpha\beta$ TCRs have been redesigned to reduce cross competition within T lymphocytes harboring naïve TCR (Kuball et al., 2007; Robbins et al., 2008).

4.1.2 Cancer Antigen Receptor (CAR)-modified T cells

Adoptive immunotherapy for cancer utilizes additional bioengineering strategies adaptable to drug resistant immunotherapy whereby T lymphocytes are genetically modified to express chimeric antigen receptors. In contrast to chimeric $\alpha\beta$ TCR, CAR combines antigen specificity derived from a tumor antigen specific monoclonal antibody fragment and T cell proliferation signal moieties. Upon infusion into patients, immunocompetent T cells genetically engineered to express CAR can specifically recognize and respond to soluble, immobilized and/or tumor antigens and, to date, a range of CARs targeting a variety of surface molecules expressed by many solid tumors and hematological malignancies, such as B cell malignancies and melanoma, have been developed (Kohn et al., 2011).

The potency of CAR modified CTLs have evolved through several generations of design changes (Cartellieri et al., 2010). First generation CARs are constructed by the fusion of the single-chain Fv (scFv) moiety, derived from the light and variable chains of a monoclonal antibody, directed against tumor associated antigens with the transmembrane and cytoplasmic signaling domains derived from the CD3 ζ chain. The CD3 domain provides activation signal to the CAR for the induction of cytotoxicity towards the tumor expressing the protein that is recognized by the scFV. Thus CAR integrates the antigen specificity of an antibody and anti-tumor properties of CTLs. However, bioengineered CTLs were poorly activated by the first generation CARs, possibly due to insufficient co-stimulatory signaling as evident by low response rates in clinical trials in subjects with various malignancies, such as lymphoma, and ovarian cancer (Kershaw et al., 2006; Lamers et al., 2006). To circumvent this issue, various signaling domains from costimulatory molecules such as CD28, OX40, and CD137 (4-1BB) were fused to the cytoplasmic tail of the CAR which improved the anti-tumor efficacies of the CTLs modified with the second generation CAR in preclinical models (Kowolik et al., 2006). To further enhance the potency of the engineered CTLs, recent third generation CARs are designed to incorporate tripartite signaling domains, such as CD3 ζ -CD28-41BB or CD3 ζ -CD28-OX40. Several vector systems have been designed to introduce the chimeric receptors into T cells. Such systems include γ -retroviral vectors, lentiviral vectors and transposon based (sleeping beauty) constructs (Hackett et al., 2010; Westwood & Kershaw, 2010).

CAR based anti-neoplastic cellular therapy can be applicable to patients with any HLA type since CARs use antibodies as the component that recognize the target antigen and thereby the CAR-modified cells act in "HLA non-restricted" fashion to destroy their target. Thus CAR mediated cellular immunotherapy is refractory to the immune evasion strategies by tumors, such as downregulation of HLA class I molecules or failure to process or present proteins. However, CAR can be targeted only against extracellular (surface) antigens, which represent only a subset of potential tumor-associated antigens. To circumvent limitation, CARs have also been designed to recognize carbohydrates and glycolipids (Dotti et al., 2005; Sadelain et al., 2009). It should also be noted that currently, murine derived antibodies are employed to design the antibody components of most CARs, which raises the possibility of evoking immune responses against the CAR-engineered cells after infusion.

4.2 MHC-unrestricted immune cells

Conventional cell based immunotherapeutic strategies that are based on the activation of HLA-restricted lymphocytes have limited anti-tumor response. This is due to i) frequent down regulation of HLA on the tumor cell surface thereby mitigating the activity of adaptive immune responsive cells, ii) secretion of immunosuppressive factors by the tumors and iii) limited expression of tumor antigens in small subset of the tumor cells. Thus novel strategies that harness the anti-cancer responsive-innate immune cells, such as NK92 cells and a minor subclass in the T lymphocyte repertoire, $\gamma\delta$ T cells present a promising alternative to conventional adaptive cell based therapy approaches to treat cancer.

4.2.1 Natural killer (NK) cells

NK cells comprise a unique subset of lymphocytes, distinct from T and B cells, and are members of the innate immune response cells with potent immunosurveillance properties. These cells do not require any prior immune sensitization by the host to lyse tumor cells

(Herberman et al., 1975; Kiessling et al., 1975; Klingemann, 2005). Early pioneering work demonstrated the therapeutic benefits of adopting innate immune responsive killer cell based strategies, specifically with LAK cells along with IL2, to target advanced metastatic renal cell carcinoma and melanoma (Rosenberg, et al., 1985). However, later studies found similar benefits with administrations of IL2 alone (Law et al., 1995). It was initially thought that NK cells exhibit potent cytotoxicity towards transformed cells that express altered MHC molecules (missing self recognition) while sparing normal cells that express unaltered MHC molecules (self recognition) via the activation of the inhibitory receptors. However, NK cells are able to efficiently attack some target cells that express normal levels of class I MHC molecules, while some other cells are not sensitive to NK cell-lysis despite low or absent class I MHC expression. It is now established that NK cells express NKG2D (natural killer group D) receptors that are activated by the recognition of ligands that are strongly upregulated in stressed tumor cells (Bauer et al., 1999; Cosman et al., 2001). Surprisingly, normal non-stressed cells of bone marrow activated peripheral blood T lymphocytes and even normal non-hematopoietic cells express NKG2D ligands. The specific roles of NK cells towards each of these cell types are under investigation (Eagle et al, 2009). The use of autologous NK cells has met with limited success (Burns et al, 2003; Law et al, 1995; Rosenberg et al, 1985). Consequently, focus has shifted to the use of allogeneic NK cells to treat cancer (Miller, et al., 2005; Ljunggren & Malmberg, 2007).

Several NK cell lines have been developed that share functional and phenotypic characteristics of activated NK cells. Among these, the most promising is the NK92 cell line, an allogeneic cell line derived from a patient with non-Hodgkin's lymphoma (Gong et al., 1994; Tam et al., 1999; Tonn et al., 2001). There are several advantages to employing NK92 cells, or similar cell lines, in adoptive immunotherapy: i) they represent a well characterized immunophenotype with powerful anti-tumor properties that are independent of MHC restrictions, ii) these cells express activating receptors and lack most of the inhibitory killer immunoglobulin-like receptors, KIRs (Middleton et al., 2002), thus retaining their cytotoxicity against cancer cells that up-regulate MHC class I molecules and iii) the ease of culturing these cells to generate adequate quantities for clinical use. Currently, there are several clinical trials underway to evaluate the efficacy of NK cell mediated cancer immunotherapy.

4.2.2 $\gamma\delta$ T cells

$\gamma\delta$ T cells are defined by their expression of T cell receptors (TCR) encoded by γ and δ loci. $\gamma\delta$ T cells combine features of both innate and adaptive immune systems. These cells exhibit direct anti-tumor properties via MHC-independent NKG2D and TCR pathways. $\gamma\delta$ T cell based immunotherapy strategies have been extensively tested to target GBM (Bryant et al., 2011; Lamb, 2009). Thus, $\gamma\delta$ T cells that require no priming and mediate their cytotoxicity by direct recognition of chemotherapy induced antigens on the surface of GBM cells represent an attractive cellular immunotherapeutic candidate for GBM therapy. In this context, genetic engineering of $\gamma\delta$ T cells that are resistant to temozolomide, the frontline chemotherapy agent to treat GBM, presents an attractive scenario whereby drug resistant $\gamma\delta$ T cell based immunotherapy can be administered in combination with a traditional chemotherapeutic agent.

Freshly isolated and expanded $\gamma\delta$ T cells from the peripheral blood of healthy donors can destroy neuroblastoma cells while adoptive transfer of $\gamma\delta$ T cells, expanded under clinical

grade conditions, in combination with immunocytokines are effective against disseminated neuroblastoma established in mice (Otto et al., 2005; Schilbach et al., 2000). Recently, a clinical study of 25 patients with advanced stages of various solid tumors demonstrated that $\gamma\delta$ T cell based immunotherapy is beneficial and importantly, such a therapy did not induce any serious treatment related side effects (Noguchi et al., 2011). However, these cells comprise a minor fraction (1-5 %) of the peripheral blood lymphocytes and consequently $\gamma\delta$ T cell based immunotherapy requires prior expansion *ex vivo*. Protocols to expand $\gamma\delta$ T cells using therapeutic grade materials have been developed to facilitate the initiation of clinical trials to treat patients with cancer (Noguchi et al., 2011).

5. Conclusion

Although the combination of surgery and chemotherapy is effective for some types of cancer, there are obvious limitations to our current state-of-the-art treatment of cancer. Among the various potential therapeutic modalities being used to treat cancer, immunocompetent cell-based therapy is becoming an effective alternative, which is possible because of the advancements in technologies used to genetically engineer these cells. As described in this chapter, various engineering strategies have potentiated the acquired or intrinsic anti-tumor response of immunocompetent cells. It is now anticipated that bioengineered cell-based therapies, when partnered with conventional chemotherapies, can invoke an anti-tumor response that is superior to results achieved by the individual therapies. Both the chemotherapy and cellular therapy fields are focused on determining optimal strategies for combining these therapies, and many types of combination approaches are being evaluated. Several successes using combination strategies have already been reported. For example, administration of chemotherapy following vaccine based immunotherapy have shown therapeutic efficacies in patients with several types of cancer, such as small-cell lung cancer, prostate cancer and advanced stages of ovarian, breast, colorectal, renal and prostate cancers (Antonia et al., 2006; Arlen et al., 2006; Gribben et al. 2005). Strikingly, when chemotherapy was added to tumor bearing mice previously administered with immunotherapy, both the percentage and potency of tumor specific immunocompetent cells were increased (Fridlender, et al., 2010), indicating that immunotherapy and chemotherapy can be combined, but combining the two treatment modalities is not straightforward. Although potential synergism exists between chemotherapy and immunotherapy, drug mediated myelosuppressive effects limits the employment of immune-effector cells during chemotherapy applications. DRI can allow for the administration of a dual therapy regimen, which combines genetically engineered drug resistant cell-based therapy with chemotherapy. Proof-of-concept studies evaluating DRI have yielded promising results, which show robust anti-tumor responses can be maintained during chemotherapy challenges. However, challenges remain as to i) the manipulation of the immune effector cells, ii) the timing of infusion of the bioengineered cells with chemotherapy, and iii) the long term safety profiles of such treatments. But the potential benefits afforded that can be accomplished by employing engineered immune cells, and specifically modified cells that have been engineered as drug resistant cells, warrants the continued development of such therapeutic approaches. The use of cDNA sequences that confer drug resistance to immunocompetent cells can eventually be directed toward a broad range of human malignant diseases that continue to have unmet medical needs.

6. References

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The aim of this book is to cover key aspects of existing problems in the field of development and future perspectives in gene therapy. Contributions consist of basic and translational research, as well as clinical experiences, and they outline functional mechanisms, predictive approaches, patient-related studies and upcoming challenges in this stimulating but also controversial field of gene therapy research. This source will make our doctors become comfortable with the common problems of gene therapy and inspire others to delve a bit more deeply into a topic of interest.

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