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Adoptive immunotherapy: good habits instilled at youth have long-term benefits

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Abstract Many recent advances in basic cell biology and immunology are a harbinger of progress in adoptive cell therapy (ACT) including (1) the finding that host lymphodepletion enhances engraftment and efficacy, (2) the recognition that in vitro T cell functions may not correlate with in vivo efficacy, and (3) the development of advanced ex vivo culture methods to expand lymphocytes to therapeutically effective numbers. In this article, we focus on the development of artificial antigen presenting cells (aAPCs) in our laboratory and their applicability to augment ACT protocols. We also describe how aAPCs can be used to broaden ACT to treat patients with a wide variety of cancers, chronic infectious diseases, and autoimmune manifestations.

Keywords Adoptive cell transfer therapy · Artificial antigen presenting cells · Regulatory T cells · Th17 Cells · Central Memory T cells

Introduction

Adoptive cell transfer (ACT) is an effective therapy for patients with certain types of cancer and chronic infectious disease [1–5]. This approach involves ex vivo stimulation and expansion of autologous or allogeneic T cells followed by infusion into patients. This approach has many potential advantages including: 1) large numbers of lymphocytes ($1 \times 10^{9-11}$) can be administered to patients, 2) cells can be endowed with desired effector functions, and 3) in vivo engraftment and expansion can confer long-lasting immunity. In

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spite of these advantages, however, long-term objective responses have only been reproducibly achieved in patients with melanoma and virally induced lymphomas, and in the setting of allogeneic T cell infusions after bone marrow transplantation [6].

Many advances in basic T cell biology are shedding new light on how best to generate potent and specific lymphocytes for the immunotherapy of cancer. These advances will broaden the scope of ACT applications to treat infectious diseases and autoimmune diseases. A successful cell therapy requires (Fig. 1), at a minimum (1) proper preconditioning of the patient prior to ACT, (2) selection of the optimal stem cell or lymphocyte subset(s) for ACT applications, and (3) development of effective ex vivo expansion strategies to obtain sufficient numbers of therapeutically effective cells without compromising their effector functions or their in vivo engraftment ability.

Preconditioning the host enhances ACT treatment in patients. Early adoptive transfer trials with antigen-specific tumor-infiltrating lymphocytes (TILs) in humans yielded disappointing long-term responses [7–10]. However, in studies from the National Cancer Institute, patients with advanced metastatic melanoma who underwent a cyclophosphamide/fludarabine lymphodepletion regimen prior to adoptive transfer of TILs achieved objective response rates greater than fifty percent [11, 12]. In our laboratory, lymphodepletion regimens have been incorporated in the evaluation of combination therapy in patients with lymphoma [13] and multiple myeloma [14]. Combination therapy consisting of a single early post-transplant infusion of in vivo vaccine-primed and ex vivo costimulated autologous T cells followed by post-transplant booster immunizations induced potent immunity in the patients.

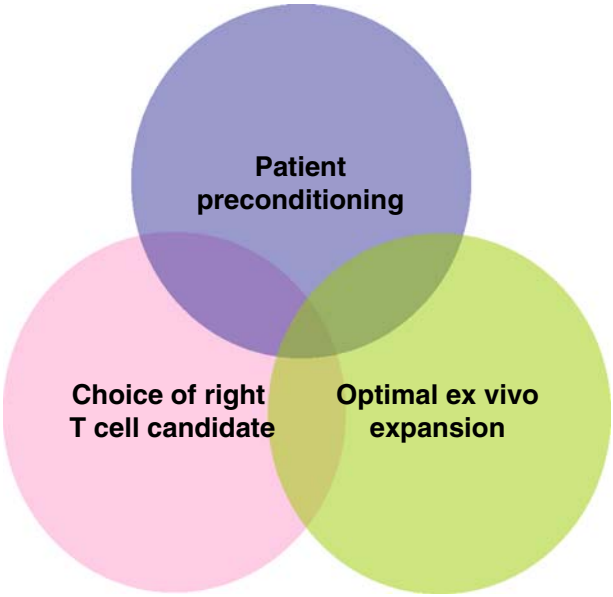

Fig. 1 Essential factors for augmenting adoptive immunotherapy. It is now clear that successful cell therapy needs to encompass at least three important factors: (1) proper preconditioning of the patient prior to ACT (i.e. surgery or various lymphodepleting preparative regimens), (2) the selection of the right cell type for programming and engineering (stem, cord blood cell, peripheral blood T cells), as well as the correct differentiation state of the cell, and (3) development of effective ex vivo culture strategies (cytokines, beads, or artificial APCs) that expand lymphocytes to unique T cell subsets

Host preconditioning is not the only factor responsible for affecting clinical responses. Emerging findings in mouse models indicate that the differentiation status of transferred cells is also important to the success of T cell-based therapies [15]. The criteria currently used to select cultured cells for infusion into patients are their ability to produce high levels of IFN-γ and their in vitro cytolytic capacity. These differentiated cells have full effector functions, as indicated by their downregulation of CD62L, CCR7, and CD27 and their upregulation of CD25 and granzymes. Surprisingly, Gattinoni and coworkers found that the most effective T cells for mediating tumor destruction were not those with a fully differentiated effector memory phenotype (T_{EM}) but rather those that retained a less differentiated central memory phenotype (T_{CM}), as indicated by higher expression of CD62L, CCR7, and CD27 [15]. Interestingly, naïve transgenic tumor-reactive T cells were found to be superior to T_{CM} cells in potentiating tumor immunity. Likewise, Berger and colleagues reported that in a nonhuman primate model antigen-specific CD8+ T_E clones derived from T_{CM} but not T_{EM} precursors are able to persist long term, migrate to T_M niches, and acquire phenotypic and functional properties of T_{CM} after adoptive transfer [16]. Collectively, these data suggested that minimally differentiated, "youthful" lymphocytes may be preferable for augmenting ACT in humans.

The focus of this article, and the long-standing focus of the June Laboratory, is the development and optimization of ex vivo T cell culture systems for adoptive immunotherapy. The laboratory has long been a pioneer in the use of artificial antigen-presenting cells (aAPCs) for "youthful" T cell expansion [17]. With an enlarging toolbox of engineered aAPCs to express virtually any costimulatory molecule or produce any type of cytokine, we are at the brink point of generating nearly any type of human lymphocyte, including CD4+ T cells with Treg, Th1, Th2, and Th17 functions, and CD8+ T cells with "stemness", central and effector memory functions. This accomplishment is made possible, in part because the cord blood cells, with their exquisite naivety, are more pliable to influences imprinted on them by cytokine and/or chemical manipulation [18]. As part of the Translational Research Program of the Abramson Family Cancer Research Institute at the University of Pennsylvania, our laboratory has made the mantra of bench-to-bedside research a reality. This is possible through our long-standing interaction with the University of Pennsylvania Cell and Vaccine Production Facility (CVPF), a good manufacturing practice (GMP) facility whose primary function is the manufacturing of cell products for T cell adoptive transfer trials in both cancer and HIV [19]. Thus, our basic research into T cell activation and proliferation has been, and will continue to be, translated to the clinic.

Generating potent T cells for the clinic

The overall therapeutic aim of our laboratory is depicted in Fig. 2. In addition to purified peripheral blood mononuclear cell subsets obtained from regular leukaphereses of healthy donors, we have established a large repository of viable cord blood cells, peripheral blood cells, TILs, and tumor cells from healthy donors or cancer patients. Thus, abundant supplies of primary human cells are readily available. In order to generate cell-based aAPCs, we have developed a lentiviral vector system that enables highly efficient and stable modification of target cells with a desired gene. This vector system, and its use in the genetic modification of T cells, is described in detail in the accompanying article by Varela-Rohena and colleagues [20]. Functional evaluation of aAPCs is performed using a wide array of in vitro assays. In addition, we have established a NOD/scid/IL-2Rγ_c^{null} (NOG) ACT mouse model [21] that permits the in vivo evaluation of engraftment and function of T cells.

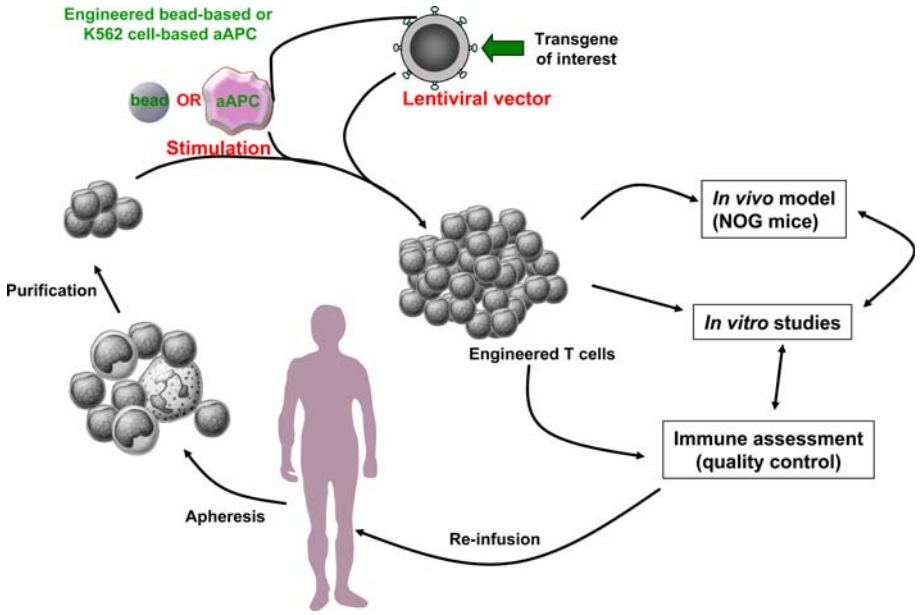

Fig. 2 Adoptive cell transfer strategy. Input cells are isolated by apheresis for example, or tumor digestion (not shown), purified, and stimulated with an artificial antigen presenting cell (aAPC). The desired phenotype can be engineered into the cells, predominantly through high efficiency lentiviral vector-mediated transduction. The cells are then rapidly expanded and subject to both in vitro and in vivo functional assays prior to infusion into the patient

This model can be used to evaluate the therapeutic potential of transferred T cells on various diseases, such as cancer, HIV, and autoimmunity. These mice lack mature B and T cells, and have virtually no NK cells. In addition to being exceptionally permissive to human leukocyte engraftment, the complete human immune system can be reconstituted in these animals following injection of human hematopoietic stem cells [21]. Performing experiments in NOG mice reconstituted with these cells will be important because it will allow for workers to gain insight into how these reconstituted cells, present in the normal human immune system, impact on the adoptively transferred human lymphocytes.

The first generation: bead-based aAPCs

Dendritic cells (DCs) are the most potent natural stimulators of the immune system and thus are ideally suited for T cell expansion [22, 23]. However, ex vivo approaches using autologous DCs to expand T cells for adoptive immunotherapy have been hampered by difficulties in obtaining large numbers of these terminally differentiated, short-lived cells. Major obstacles to the use of DCs in adoptive immunotherapy include the expense of preparing DCs, batch-to-batch variation among donors, and poor yields from in vitro cultures. Furthermore, the reported dysfunctional nature of DCs from cancer patients further complicates their use [24]. Limitations with autologous DCs prompted us to initiate the development of potent, reproducible, and GMP-compliant aAPCs over the previous decade. The evolution of these aAPCs is shown schematically in Fig. 3.

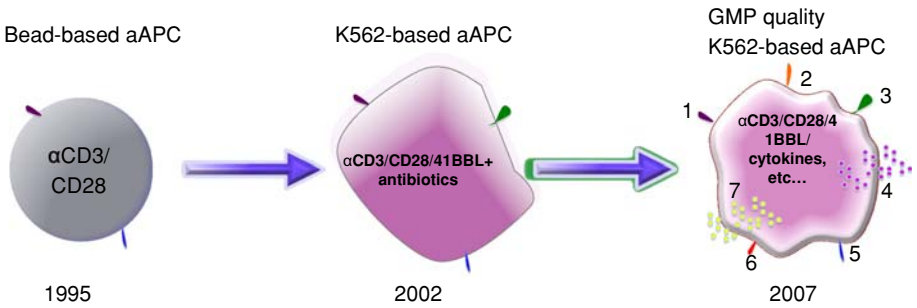

Fig. 3 The evolving artificial antigen presenting cell (aAPC). Due to the limitation of autologous DC to reproducibly expanding large numbers of quality human T cells, various types of aAPCs were developed over the past decades to improve the yield of lymphocytes obtained from patients for ACT therapy. The first generation of aAPCs consisted of antibodies to CD3 and CD28 covalently bound to paramagnetic beads. More recently, the use of cell-based aAPCs has been explored. The first generation of K562 cell-based aAPCs was produced using plasmid transfection and antibiotic selection. The most recent generation of K562-based aAPCs has been constructed by lentiviral vector mediated-transduction. High titer lentiviral vectors permit the introduction of numerous (up to 7) costimulatory molecules or soluble immunomodulators

The first generation of aAPCs developed in our laboratory consisted of antibodies to CD3 and CD28 covalently bound to paramagnetic beads. By concurrently delivering both signal one (anti-CD3) and signal two (anti-CD28), these beads directed robust proliferation of human CD4+ T cells [17]. This approach reproducibly drove multiple rounds of proliferation, resulting in greater than 1×10^9 fold expansion of the input cell population. This large expansion is due at least in part to the CD28-mediated induction of telomerase in CD4+ T cells [25]. Therefore, despite extensive ex vivo replication, anti-CD3/anti-CD28 bead-expanded cells retain extensive in vivo proliferative capacity. Furthermore, it was discovered that anti-CD3/28-coated beads also promoted vigorous expansion of CD4+ T cells from HIV-infected donors, and that during culture the number of HIV-positive cells declined to nearly undetectable levels [26, 27]. These observations led to the manufacture of GMP-compliant anti-CD3/CD28 beads and the first Phase I clinical trial conducted by our laboratory [26, 28]. Since then, antibody CD3/CD28-coated beads have been extensively used to expand CD4+ cells for use in multiple clinical trials, both at the University of Pennsylvania and other sites. Table 1 contains descriptions of a sample of the trials in both cancer and HIV-1 in which these first-generation aAPCs have been employed [14, 28–32]. To date, the CVPF has generated expanded T cell products for more than 200 patients.

Table 1 Select clinical trials using bead-based aAPCs for T cell expansion

Disease	# Patients treated	Reference
HIV	8	[26]
HIV	5	[27]
Advanced hematologic malignancies	17	[28]
Multiple myeloma	52	[29]
Non-Hodgkin's lymphoma	5	[30]
Chronic myelogenous leukemia	4	[31]
Neuroblastoma	35	Ongoing
Multiple myeloma	30	Ongoing
HIV	10	Ongoing

The second generation: K562 cell-based aAPCs

While bead-based aAPCs continue to be used in both clinical and preclinical studies, they suffer from certain limitations. First and foremost, bead-based aAPCs do not support extended proliferation of CD8+ T cells, especially in the case of human CD8+ T cells that lose CD28 expression with age, in contrast to the mouse that retains expression throughout life. Intensive efforts have long been underway to develop cell-based alternatives to the beads. The erythromyeloid line K562 was chosen as the platform for this approach. Importantly, K562 cells do not express MHC Class I or Class II proteins and thus do not drive allogeneic T cell proliferation [33]. However, they express T cell adhesion molecules such as ICAM and LFA-3 that enhance T cell–APC interactions. These aAPCs were generated by transfecting K562 cells with plasmids encoding 4-1BBL and the human Fc receptors CD32 or CD64. The costimulatory molecule 4-1BBL interacts with the TNF receptor family member 4-1BB, which is present on activated T cells [34]. Importantly, signaling through 4-1BB activates and enhances CD8+ T proliferation and function in vitro and in vivo. Inclusion of CD32 permits the exogenous loading of anti-CD3 and anti-CD28 antibodies. Using these cells, we found that flu-specific CTLs could expand exponentially for greater than two months while maintaining antigen specificity and effector function, resulting in a 10,000-fold expansion of antigen-specific CD8+ T cells [33].

Although K562 cell-based aAPCs promote the expansion of CD8+ T cells, they were generated using transfection and thus gene expression is reliant upon continued antibiotic selection, which does not meet GMP requirements. Furthermore, surface expression of introduced molecules was not stable, even in the presence of antibiotic transfection [35]. These shortcomings prompted us to generate clinical-grade cells able to stably express several costimulatory molecules. To achieve this goal, we developed a lentiviral vector system capable of high-efficiency transduction of both primary and transformed cell lines. This approach allowed for the generation of K562-based aAPCs capable of expressing multiple gene inserts, including human lymphocyte antigen (HLA)-A2, CD32 (the low-affinity Fc receptor), CD64 (the high-affinity Fc receptor) CD80, CD83, CD86, CD137L (4-1BBL) and CD252 (Ox40L) [35] among others. The expression of multiple genes on the aAPCs aided our understanding of the basic requirements for T cell activation. In contrast to bead-based aAPCs, these GMP-quality K562-based aAPCs supported the long-term expansion of functional human CD8+ T cells, efficiently expanded genetically modified T cells and maintained CD28 expression on human CD8+ T cells. Finally, the costimulatory ligands on the aAPCs enable efficient proliferation and expansion of CD8+ T cells without the need of exogenous cytokines or feeder cells as used in the current cell culture processes. The replacement of CD32 with CD64 added several important clinically relevant features to these aAPCs. First, the tight binding of antibodies to CD64 enables extensive washing of the cell product, thus reducing the potential for infusion of murine antibodies and generation of a human anti-mouse antibody immune response. Secondly, antibody-loaded CD64-expressing K562 cells can be cryopreserved, thawed, and used, with no loss of function, thus permitting even greater standardization of aAPC lots. GMP-compliant master cell banks of K562 aAPCs are being evaluated and characterized and will soon enter Phase I clinical trials. Undoubtedly, these aAPCs have the therapeutic potential for impacting on the next generation of T cell-based therapies [36].

The next generation: tumor cell-based aAPCs?

While K562-based aAPCs remain the laboratory workhorse to study the basic principles of T cell biology, we have recently initiated an effort in developing a toolbox of tumor cell-based aAPCs. This effort is a direct outcome of our development of methods by which stable cell lines can be established from primary tumors at reasonably high efficiencies [37]. For this purpose, we have been archiving viable primary tumors and lymphocytes, primarily from patients with lung cancer, ovarian cancer, and mesothelioma. Tumor cells present some attractive features as aAPCs. In addition to their ability to self-renew, they are easily maintained resources, and they can present the entire tumor-associated antigen repertoire in an MHC-restricted fashion [38]. Tumor-associated antigens run the spectrum from unique to universal (shared among many if not all tumors). Examples of the latter include telomerase and survivin [39, 40]. Since it is presently unclear whether responses directed at private or universal antigens will most effectively eradicate tumors [41, 42], presentation of a diverse array of tumor-associated antigens may be prudent as they might induce immune responses.

There are numerous characteristics inherent to tumor cells that would seem to preclude their use as antigen-presenting cells. In addition to producing suppressive cytokines such as TGF-β and IL-10 [43], they are poorly immunogenic. They can induce anergy or tolerance based on MHC I-restricted antigen presentation in the absence of costimulation. However, the advent of lentiviral vector technology and other approaches for genetic engineering [44], combined with our ever-expanding "molecular toolbox", provides us with the opportunity to generate robust tumor-based aAPCs. In fact, the introduction of costimulatory molecules and other immunomodulators in tumor cells has been shown to enhance tumor immunogenicity [45]. Our strategy for generating tumor-based aAPCs is similar to generating K562-based aAPCs, i.e. introduction of multiple costimulatory molecules and soluble immune modulators into the tumor cell lines. We are currently assessing the ability of modified tumor aAPCs to stimulate both antigen-specific CD8+ T cell proliferation (using influenza peptides as a model antigen) versus bulk proliferation of cells isolated from the peripheral blood or malignant effusions. Furthermore, we are evaluating the function of tumor aAPC-stimulated CD8+ cells using in vivo humanized ACT mouse models.

Programming human T cell subsets with aAPCs

Current evidence suggests that naïve CD4+ cells are instructed to differentiate into distinct subsets based on the contextual signals delivered during antigen presentation. To test this hypothesis, we have created a library of aAPCs to determine the optimal costimulatory signals and cytokines required to foster the expansion of functionally active human Tregs, Th1, Th2, and Th17 cells for augmentation of ACT therapies (Fig. 4).

Expanding human Tregs with aAPCs

CD4+CD25+Foxp3+ regulatory T cells (Tregs) were initially described as a cell population important for the control of autoimmune diseases [46]. While cancer immunotherapists view Tregs as a cell subset to be eliminated or at least neutralized [47–49], the potent tolerizing properties of Tregs have numerous potentially beneficial clinical applications, such as prevention of graft-versus-host disease after allogeneic bone marrow transfer, as well as allogeneic tolerance following solid organ transplants [50]. However, clinical-scale Treg

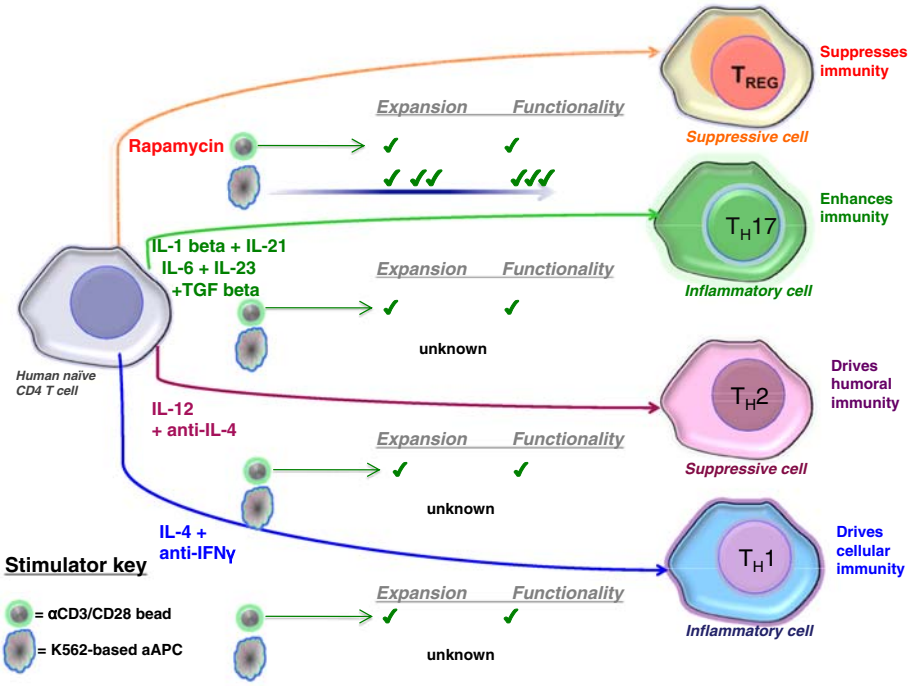

Fig. 4 Development of artificial APCs that program human CD4 T cells to a Treg, Th17, Th1 or Th2 phenotype. Naïve CD4 T cells can be polarized by modulating cytokines, costimulatory molecules or signaling pathways such as mTOR using rapamycin. Bead-based aAPCs promote the expansion of the polarized CD4 T cells. With the exception of Tregs, the ability of K562 cells to promote expansion of functional, polarized Th1, Th2, and Th17 cells remain largely unexplored

expansion poses technical challenges. For example, peripheral blood Tregs are scarce, and it is difficult to obtain pure Treg populations due to the present lack of defining cell surface markers. Furthermore, compared to bulk CD4+ T cell populations, Tregs are at a replicative disadvantage under most ex vivo culture conditions [51]. Our laboratory has conducted a systematic exploration of the costimulatory requirements for Treg expansion. We generated a series of K562-based aAPCs designed to provide costimulation through CD28, CD27, OX40, or 4–1BB signaling pathways [52]. We found that only CD28 costimulation, in the presence of inhibition of the mTOR pathway by rapamycin, promoted the expansion of Treg populations that retained functional capacity. Furthermore, we demonstrated that under these conditions and using two amplification cycles, 1,000-fold expansion of the starting cell population could be achieved. To analyze their function in vivo, we developed a xenogeneic graft-versus-host disease (xGVHD) model using NOD/*scid*/IL-2Rγ_c (NOG) mice. When implanted with bulk human PBMCs, these animals develop lethal xGVHD in 6–8 weeks. Addition of ex vivo expanded Tregs significantly delayed xGVHD onset. It is important to note that rapamycin was not administered to the animals, demonstrating that the ex vivo culture conditions endowed the Tregs with a stable suppressor phenotype.

While the observation that the addition of rapamycin to murine Treg cultures enhances the Treg yield have proven to be a fundamental advance in ex vivo Treg culture [53], it was unclear how rapamycin maintains the Treg suppressor phenotype. In fact, one study suggested that rapamycin conferred a transient Treg-like state upon CD4+ effector cells [54].

This raised an important clinical issue, as presumably these "pseudo-Tregs" would revert to their native effector phenotype in vivo upon rapamycin withdrawal. To resolve this issue, we transduced CD4+CD25- T cells with lentiviral vectors encoding the Treg master regulator Foxp3 [46] and demonstrated that the transduced cells were selectively enriched when expanded in the presence of rapamycin. Furthermore, forced FoxP3 expression resulted in expression of the serine-threonine kinase pim 2, which has been shown to mediate resistance to rapamycin [55]. By elucidating that pim 2 was constitutively expressed in high purified resting Tregs, we demonstrated its importance in Treg function [56]. These observations indicate that Foxp3-mediated constitutive expression of pim 2 confers a growth advantage on Tregs in the presence of rapamycin. Therefore, rapamycin acts to positively select for Treg expansion in a pim 2 dependent manner, a finding that might have important implications in adoptive immunotherapy for patients with various autoimmune diseases.

Expanding human Th1, Th2, Th17 cells with aAPCs

Human CD4+ T cells can differentiate into multiple subsets but the potential roles of these subsets in antitumor immunity have been incompletely elucidated. Studies from our laboratory and others indicate that human CD4+ cells retain more plasticity after antigen priming than their mouse counterparts [57, 58]. Given the superb capacity of aAPCs to effectively expand and greatly preserve the suppressive functionality of human Treg cells when cultured in rapamycin, it might be possible that aAPCs can be designed to specifically promote the growth of functional human Th1, Th2, and Th17 cells for various adoptive immunotherapeutic approaches (Fig. 4).

Th1 cells have long been recognized to potentiate antitumor and antiviral immunity [59, 60]. Thus, as shown in Fig. 4, aAPCs could be designed that program antigen-specific lymphocytes toward the Th1 subset. These expanded cells can then be tested for their capacity to eradicate tumors in our humanized ACT model. Therefore creating aAPCs that produce IL-12 or IL-4 (cytokines that confer Th1 and Th2 function, respectively) might selectively expand CD4+ T cells to these particular subsets [61]. We previously showed that qualitative alterations in CD28 signaling could lead to changes in Th1 or Th2 bias in mouse CD4+ T cells [62].

In contrast to the current view that Th1 cells play the most important role in tumor rejection, preclinical experiments recently revealed that transgenic Th17-polarized cells were superior in mediating destruction of large tumors in mice [63]. Furthermore, Th17 cells were found to mediate greater tumor regression than Th1 or Th2 cells. Although Th17 cells mediate superior tumor immunity compared with the other cell subsets in mice, the therapeutic potential of Th17 cells in enhancing ACT therapy remains unknown. Using our humanized ACT tumor mouse model, we could determine whether human Th17 cells are more effective in augmenting tumor immunity than human Th1 or Th2 cells. The findings from these experiments will be insightful in guiding future T cell-based therapies in the clinic.

Substantial basic biology on how human Th17 cells impact human diseases has rapidly unfolded and the cytokines which program CD4+ T cells to inflammatory Th17 cells have been clearly defined [64–69]. Sallusto and coworkers first found that IL-1-β fosters the development of human CD4+ cells that produce IL-17 [70]. The addition of IL-6 in the culture increased IL-17 production by these cells. Recently, the Littman group revealed that TGF-β is necessary for generating Th17 cells [71]. Furthermore, cytokines IL-21 and IL-23 were found to play an important role in programming human CD4+ T cells to Th17 cells. Thus, K562-based aAPCs constructed to generate TGF-β, IL-1-β, IL-6, IL-21, and IL-23 might profoundly bolster the expansion and functionality of human Th17 cells. Alternatively, human

CD4+ T cells can be transduced with the transcription factor RORC or RORA to confer Th17 function [72] and, perhaps, expanded with "Th17 aAPCs" to sustain their long-term growth.

Given the recent findings that Th17 cells exacerbate autoimmune responses [73], they might be ideal cells for driving immunity to tumors. Thus, it will be important to determine whether K562-based APCs modified to produce IL-1β, IL-6, IL-21, IL-23, and/or TGF-β can efficiently program and expand human tumor-reactive CD4+ cells towards a Th17 function. Perhaps most importantly, it will be important to compare these cells to tumor-reactive Treg, Th1, or Th2 cells. Furthermore, it will be interesting to understand how these different subsets might also affect the proliferative capacity and the function of CD8+ T cells that have been redirected with antigen specificity. The findings discovered through these explorations should be taken under consideration in the design of future clinical trials involving adoptive transfer-based immunotherapy of human malignancies, chronic infectious diseases, and autoimmune disorders.

Cord blood T cells: right candidate for gene transfer?

In addition to developing aAPCs that expand CD4+ T cells to a desired subset, it is important to design aAPCs that expand CD8+ T cells possessing a preferred phenotype. For adoptive immunotherapy, the repetoire of lymphocytes from which CD8+ T cells can be derived includes naive as well as antigen experienced memory T cells. The later cells can be divided into central (T_{CM}) and effector memory (T_{EM}) subsets. These subsets vary in their homing, phenotypic and functional capacity. CD8+ T_{CM} express CD62L and CCR7, which promote trafficking into lymph nodes and proliferate rapidly upon recognition of its cognate antigen. In contrast, CD8+ T_{EM} lack CD62L, which facilitates their homing to peripheral tissues and allows them to display immediate effector function. Upon antigen recognition, both CD8+ populations proliferate and differentiate into $CD62L^{-}$ cytolytic effector T cells that express high levels of granzymes and perforin but are thought to have a limited replicative potential. Thus, acquisition of a full effector phenotype during culture has been suggested as a major reason for the poor survival of transferred T cells in mice. In mice, tumor-reactive T_{CM} cells are superior in promoting tumor eradication compared with T_{EM} cells [15], suggesting that they might be important for treating patients in the clinic.

T cell memory persists for life in the normal hosts, signifying that some T_M cells may have the ability to self-renew after differentiating to T_E in response to repeated antigen exposure. T_{CM} and T_{EM} have distinct phenotypic and functional properties, but it is unknown whether T_E cells derived from each of these T_M subsets retain any intrinsic properties of the parental cell. Using a nonhuman primate model relevant to human translation, the Riddell lab sought to determine whether T_E clones derived from purified T_{CM} or T_{EM} differed in their ability to persist in vivo or established T cell memory after adoptive transfer [16]. They found that antigen-specific CD8+ T_E clones derived from the T_{EM} subset of T_M survive in the blood for only a short duration after adoptive transfer, fail to home to lymph nodes or bone marrow, and do not reacquire phenotypic markers of effector memory T cell subset. By contrast, T_E clones derived from T_{CM} persist long term after adoptive transfer, migrate to T_M niches, reacquire phenotypic properties of T_M, and respond to antigen challenge.

Due to these important findings in mice and primates, it will be important to combine our lentiviral vector systems with novel ex vivo culturing methods to create naive or T_{CM} human lymphocytes with exquisite antigen specificity. In creating this desired T cell it will be important to choose a cell candidate that retains the greatest degree of naivety upon rapid expansion. Because cord blood T cells and stem cell precursor T cells are more naïve

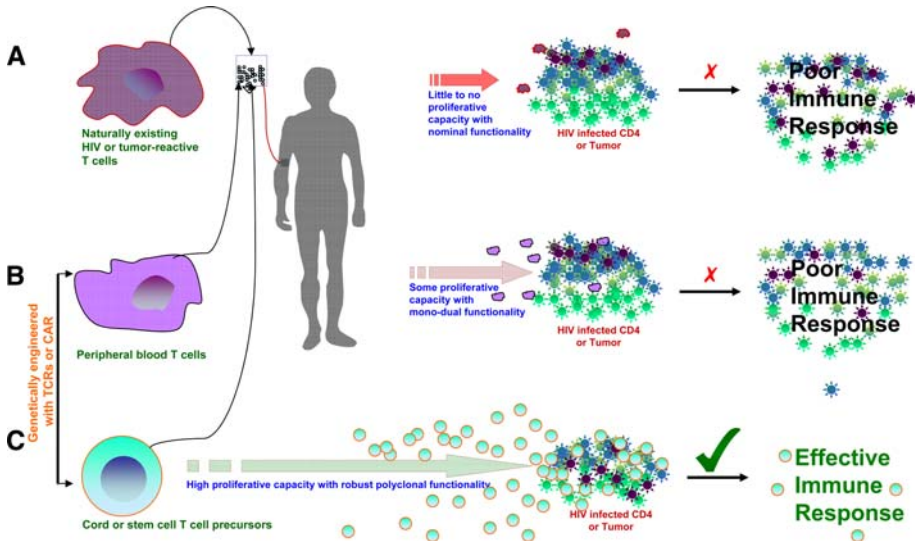

Fig. 5 Cord blood or precursor stem T cells: Greater potential for adoptive cellular transfer? Because cord blood T cells and stem cell precursor T cells are more naïve in phenotype and function compared to peripheral T cells, future adoptive transfer protocols may exploit their larger reserves of proliferative potential to enhance treatment outcome and promote life long immunosurveillance

in phenotype [74–76], even after extensive expansion, than peripheral T cells or tumor-infiltrating lymphocytes, they might be ideal candidates for driving superior antitumor or antiviral in vivo, as depicted in Fig. 5. Thus, expanded cord blood T cells and stem cell precursor T cells might retain a greater central memory signature than expanded peripheral T cells. Our laboratory has successfully transduced umbilical cord blood T cells with receptor specificity against B cell lymphomas and expanded them to large numbers for adoptive immunotherapy [77–79]. The in vivo adoptive transfer of these genetically engineered T cells significantly reduced tumor growth and prolonged the survival of the animal. Taken together, these data reveal that T cells from cord blood can be stably modified using a gene transfer systems cultivated in our lab, and that such modified T cells may be useful in the treatment of refractory leukemia and lymphoma.

Conclusion

Broadening the utility of the ACT approach will not only require genetic modification of lymphocytes, but will also require that these cells are optimally cultivated or "programmed" to subsets and lineages that enhance ACT treatment in patients with cancer, autoimmunity, or chronic infectious disease. Although less-differentiated lymphocytes mediate superior antitumor immunity compared with fully differentiated lymphocytes in mice, it remains unclear what lineage or subsets might best impact on the treatment of cancer, autoimmunity, or chronic infectious diseases in humans. Fortunately, the impact of various human CD4+ and CD8+ subsets in tumor immunity and autoimmunity can now be further understood because of recent advances in ex vivo culture methods developed in our laboratory, which allow for the expansion of human central and effector memory CD8+ cells as well as various CD4+ T cell subsets (i.e. regulatory T cells as well as Th1, Th2, and Th17 cells). How each

human subset is expanded using our novel ex vivo culture systems and their influences on immune responses will provide vital information on how to build on the next generation of cellular therapies to regenerate and augment immune system function.

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