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4. Bone marrow examination or surgical excision or biopsy of the suspected lesions

Treatment

Children with cancer are best cared for at a pediatric comprehensive cancer care center that includes a team of pediatric oncologists, nurses, surgeons, radiation oncologists, pathologists, and supportive care services for the patients and their families. Treatment includes a combination of chemotherapeutic agents with or without local radiation therapy and surgery. Chemotherapy is usually given intravenously, intraspinally, intramuscularly and by mouth. The specific agents depend on the disease being treated based upon 40 years of clinical trial experiences. Side effects include hematological toxicities (anemia, neutropenia and thrombocytopenia), nausea and vomiting, alopecia, infections, cardiac, renal and liver toxicities depending on the chemotherapeutic agents used. Response to treatment is assessed during therapy and treatment is adjusted based upon tumor response and patient side effects.

Outcome and Late Effects

Improved childhood cancer care including the development of combination of chemotherapeutic regimens, supportive care guidelines to minimize infection and bleeding resulted in a dramatic improvement in survival and cure of most childhood cancers and a corresponding drop in the mortality rate (childhood cancer; Fig. 1). However, physicians have become aware of late side effects to therapy that became apparent as early as weeks following completion of treatment up to several decades later. Some of these side effects were related to the cumulative dose of chemotherapy agents and resulted in dose modification or replacement in newer regimens to minimize immediate and late toxicity without affecting efficacy. Similar modifications have been made with radiation therapy by instituting techniques to improve delivery of radiation to the tumor and minimizing damage to neighboring healthy tissues (involved field radiotherapy) as well as reducing the total dose of radiation delivered.

Currently, several pediatric oncology centers have established clinics for childhood cancer survivors where they are followed for life. Patients are given advice about their risks of developing secondary cancers, long term organ toxicities (cardiac, pulmonary, neurological, hepatic and renal), fertility and psychosocial late effects.

Newer clinical trials are underway and will provide more information about childhood cancer survivors and their late effects.

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Chimeric

Definition

A mouse that carries a transgene in some tissues but not others; a mosaic animal.

► Mouse Models

Chimeric Antibodies

Definition

Hybrid immunoglobulins in which the original murine variable regions are preserved and the constant regions are switched for those of a human antibody to try to gain human effector functions. *Applications:* Antibody therapy when effector and other Fc-associated functions and properties are needed.

- Immunotherapy
- Diabody

Chimeric Antigen Receptor on T Cells

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Synonyms
CAR

Definition

A chimeric antigen receptor (CAR) consists of an extracellular antigen-binding exodomain, typically derived from a single-chain antibody fragment (scFv) of a monoclonal antibody (mAb), a spacer (such as an antibody Fc region), a transmembrane region, and one or more intracellular signaling endodomains, which can be genetically introduced into hematopoietic cells, such as T cells, to redirect specificity for a desired cell-surface antigen.

Characteristics

Background on Manipulating T-Cell Responses to Cancer

Adoptive transfer of tumor-specific T cells in mouse models can function as potent anti-cancer biological agents leading to elimination of established malignancies. Continued advances in tumor immunology support the premise and promise for ► adoptive immunotherapy as a treatment for human malignancies. Yet, infusion of tumor-specific T cells has only been partially successful in clinical oncology trials. Indeed, most of these trials demonstrate the safety and feasibility of infusing T cells, but with the exception of treating melanoma and chronic myelogenous leukemia (CML), only occasionally show a sustained anti-tumor effect. In contrast, infusing viral-specific T cells has successfully treated and protected patients from opportunistic diseases associated with adenovirus, CMV, and EBV. Why is it that augmenting an immune response against neoplasms by infusing tumor-specific T cells has proven more challenging than engendering an effective anti-viral response? The answer is partly due to the relative inability of T cells to recognize, via an endogenous T-cell receptor (TCR), poorly-immunogenic tumor-associated antigens (TAAs) compared with the highly-immunogenic/stimulatory viral antigens presented in the context of human leukocyte antigen (HLA). While TAAs generally have little or no expression in normal post-natal tissues outside of sanctuary sites, naturally-arising T cells are typically not reactive to tumors expressing a TAA due to immunologic tolerance. However, investigators have been able to manipulate T cells into recognizing TAA in the context of HLA molecules. This has been exploited by injection/infusion of (i) vaccines presenting TAA to overcome tolerance and stimulate T-cell immunity to tumors, (ii) tumor-specific T cells which have been culled from the patient and massively expanded in the laboratory, and (iii) T cells that have redirected specificity for tumor by genetically introducing pre-defined tumor-specific immunoreceptor genes. Clinical trials are currently evaluating all of three approaches.

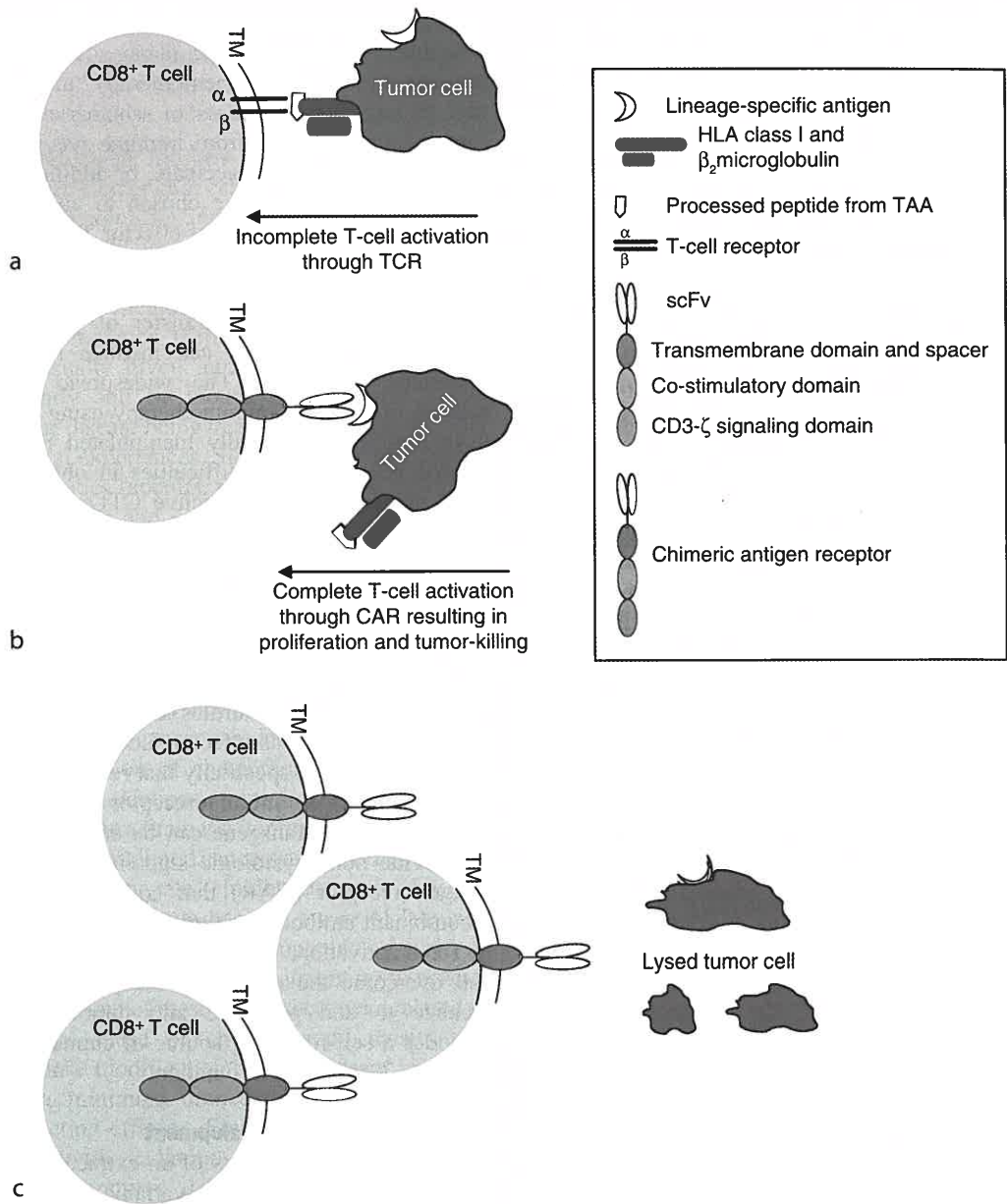
While vaccines will likely have application for cancer prevention (► cancer vaccines), vaccination to eradicate

most established tumors is a difficult challenge at this time due in part to iatrogenic damage of the underlying immune system from chemotherapy and accumulation of large tumor masses in sanctuaries which are functionally protected from immune recognition and response. Rather than vaccinate, or addition to vaccination, investigators have chosen to augment T-cell response through infusion of effector T cells (adoptive immunotherapy). Indeed, isolation, *ex vivo*-expansion of autologous tumor-infiltrating cytotoxic T-lymphocytes (CTLs), and subsequent transfer of these CTLs in lymphodepleted patients can mediate regression of metastatic melanoma. The widespread therapeutic success of adoptive immunotherapy using T cells that have not been genetically manipulated is, however, limited because of (i) difficulties in obtaining sufficient number of tumor-reactive CTLs from patients, (ii) TAAs are typically poor immunogens, (iii) existence of immune-regulatory mechanisms that prevent T-cell dependent reactivity against TAA (such emergence of tumor escape variants with loss of HLA), and (iv) the requirement that patients have pre-existing tumor-reactive cells that can be expanded *ex vivo*.

To overcome these hurdles investigators have combined the endogenous effector function of CTLs with re-directed antigen specificity that results from genetic introduction of an immunoreceptor. This introduced immunoreceptor transgene can be engineered *ex vivo* to provide non-physiologic signaling via a chimeric antigen receptor (CAR) that co-opts the ability of recombinant antibody to bind to tumor targets leading to T-cell activation. The development of CAR⁺ T cells can overcome the relative inability of antibodies to localize to and penetrate into tumor masses and provides a self-renewing source of chimeric antibody linked to T-cell effector function.

Tumor-Specific CAR Development

Generally, a CAR consists of an extracellular domain composed of a single chain variable fragment (scFv) derived from a monoclonal antibody (mAb) against a cell-surface tumor-antigen which is typically a lineage specific molecule, such as CD19 on B cells. The scFv is typically suspended from the cell surface by a spacer (e.g. mAb Fc region) and uses a transmembrane (TM) region (e.g. from CD4 or CD28) to affix the scFv-Fc to the cell surface. This TM region is in turn fused in frame to one or more signaling modules that are normally present in an endogenous TCR signaling complex, such as the CD3- ζ chain (Fig. 1). The CAR can confer scFv-mediated antigen-recognition to T cells that is independent of HLA on tumor cells and endogenous TCR. T cells genetically modified to express a CAR can be propagated *in vitro* and demonstrated to exert robust CAR-dependent effector function. Upon antigen-mediated cross-linking of the CAR, the intracellular



Chimeric Antigen Receptor on T Cells. Figure 1 (a) Incomplete activation of T cells recognizing TAA through αβ TCR in context of HLA class I. (b) Fully-competent activation signal by T cells recognizing lineage-specific antigen (e.g. CD19) independent of HLA by introduced CAR. (c) Complete CAR-mediated activation results in T-cell proliferation and tumor lysis.

signaling domain or domains initiate cellular activation which can result in proliferation, cytokine secretion, and specific cytolysis of the antigen-expressing target cells. Several requirements need to be met to enable T cells expressing CAR to exert a pronounced therapeutic effect. These include, (i) expression of the CAR at sufficient density to activate effector function upon binding antigen, (ii) generation of clinically-meaningful numbers of T cells suitable for infusion, (iii) traffic T cells to and within tumor stroma, and (iv) conditional

activation upon antigen binding, manifesting appropriate T-cell effector mechanisms such as cytokine secretion, proliferation, and cytolysis leading to tumor destruction. We and others have generated a panel of CARs and demonstrated that T cells (and NK cells) equipped with CAR mediate a highly efficient anti-tumor immune response against antigen-defined tumor target cells *in vitro* as well as *in vivo*. These CAR-grafted T cells, thus are appealing candidates for adoptive immunotherapy.

Clinical Application of CAR-Specific T Cells

Adoptive transfer of T cells for treatment of tumors is an attractive therapeutic option as it has the potential to cure disease refractory to conventional therapies. Successful allogeneic hematopoietic stem-cell transplantation (HSCT) with engraftment of donor derived tumor-specific T cells and adoptive transfer of T cells genetically rendered specific for melanoma antigens currently provide the two cornerstones for the rational application of adoptive transfer of T cells genetically modified to express CAR. Along with other investigators, we have chosen to develop CD19-specific CAR, since the CD19 molecule is widely expressed on most cancers arising from B cells. B-cell tumors are as a class poorly immunogenic with few described TAA, thus isolating and expanding endogenous T cells with specificity for malignant B cells has proven difficult in the context of allogeneic HSCT and near-impossible in the autologous setting. Thus, to target B-lineage neoplasms, an initial CD19-specific CAR (designated CD19R) was generated from the variable regions of a mouse mAb specific for CD19, and T-cell activation was achieved through chimeric CD3- ζ endodomain. The CD19 molecule is a 95-kDa membrane glycoprotein, found on human B lymphocytes at all stages of maturation, although it typically disappears upon differentiation to terminally differentiated plasma cells. It is expressed on B-lineage acute leukemias and lymphomas as well as chronic lymphocytic leukemia and is rarely lost during the process of neoplastic transformation. It is not expressed on hematopoietic progenitor cells or on normal tissues outside the B-lineage and is not thought to be shed into the circulation. An advantage of CD19-directed therapy for lymphomas over targeting CD20, another B-lineage antigen, is that unbound rituximab (CD20-specific therapeutic mAb) will not interfere with binding of CD19-specific T cells. A clinical-grade DNA plasmid vector coding for CD19R and bifunctional hygromycin (Hy) phosphotransferase selection gene fused to thymidine kinase (TK) suicide/imaging fusion gene (HyTK) was developed and produced by the National Gene Vector Laboratory. Using this vector, a gene therapy trial was opened (BB-IND 11411, ClinicalTrials.gov Identifier: NCT00182650) to determine the feasibility and safety of infusing autologous T cells co-expressing CD19R and HyTK transgenes, along with exogenous low dose recombinant human IL-2 (as a surrogate T_h-response) in patients with refractory lymphoma.

Improved Therapeutic Potential of CAR⁺ T Cells

Ongoing projects in our laboratory to improve therapeutic efficacy have focused on prolonging the survival of the infused CAR⁺ T cells mainly *via* three approaches.

1. *To produce T cells those are capable of endogenous IL-2 production.* To generate an effective anti-tumor response for CD19-redirected effector T cells in tumor microenvironment we introduced a chimeric CD28 T cell co-stimulatory molecule into the CD19R CAR. This is based on the rationale that T-cell binding of CD28 to B7 molecules on target cells generates critical regulatory signals necessary for full T-cell activation and preventing T-cell apoptosis after CAR engagement. However, massively *ex vivo*-expanded genetically modified T cells may lose endogenous CD28 cell-surface expression. Therefore, to provide genetically modified CD28^{neg} T cells with tandem activation and co-stimulation upon engagement with B7^{neg}CD19⁺ B-lineage tumors, the CD19R CAR was modified to include CD28-signalling domain. This second generation CAR, designated CD19RCD28, has been expressed in primary T cells and shown to activate genetically modified T cells for killing, IFN- γ , and IL-2 cytokine production, and improve *in vivo* survival of adoptively transferred CD19RCD28⁺ T cells resulting in a greater anti-tumor effect, compared with first-generation CD19R⁺ T cells.
2. *To target IL-2 to the tumor micro-environment.* In addition to engineering CAR for T-cell production of IL-2, a cytokine that *ex vivo*-expanded T cells typically depend on for continued *in vivo* persistence, we have directed exogenous IL-2 cytokine to the B-cell tumor microenvironment using a CD20-mAb fused to IL-2 (immunocytokine), and this combination immunotherapy enhanced the anti-tumor effect of infused T cells.
3. *To produce T cells with improved biologic potential.* Shortening the *ex vivo* manufacturing time may improve therapeutic efficacy, as extensively propagated T cells differentiate *in vitro* into cytolytic effectors lacking desired homing receptors and a tendency to undergo replicative senescence. Therefore, we have developed a new rapid propagation technology using a CD19⁺ immortalized artificial antigen presenting cell (aAPC) that can be lethally irradiated and used in co-culture to numerically expand cytolytic CD19-specific T cells. To develop non-invasive biomarkers and to evaluate adoptively transferred T-cell distribution and function, we have used radionuclides that are metabolized by TK which acts as a reporter gene for detection of T cells by positron emission tomography (PET).

Future Challenges

There has been a paucity of published data on the safety and feasibility of infusing T cells expressing CAR and at this time there are no reports describing sustained clinical response of adoptive transfer of CAR⁺ T cells. This will soon change as multiple clinical trials are currently

underway world-wide using adoptive cellular immunotherapy with T cells expressing CAR. To maximize therapeutic efficacy, future trials will likely infuse CAR⁺ T cells combined vaccine to deliver a T-cell activation signal though endogenous $\alpha\beta$ TCR, or CAR⁺ T cells combined with cytokine, such as immunocytokine, to deliver co-stimulatory signal through endogenous cytokine receptors. One of the major advantages of the strategy infusing CAR⁺ T cells lies in the modular composition of the CAR molecule that combines an antigen-binding domain with signaling domains for effector-cell activation. This allows investigators to swap the CAR exodomain with a scFv or ligand that recognizes or binds to a desired cell-surface antigen. Future experiments will build on the catalog of antigen-targeting receptors to evaluate expression level, density, and stability of the CAR expression on the T-cell surface, as well as affinity of the binding domain for antigen in the context of tumor targets with varying antigen density. These studies will impact CAR-mediated immunotherapy since low-density antigen-positive tumor cells or low affinity CAR may lead to emergence of tumor escape. Just as genetic engineering can be used to alter the CAR exodomain, so the transmembrane, or endodomain may be altered to provide a fully-competent antigen-dependent T-cell activation signal. The majority of the CARs generated harbor CD3- ζ signaling chain which is currently considered more efficient in activating T cells for cytolysis, compared to chimeric Fc ϵ RI- γ . Other CARs have been generated activating T cells through syk, lck, but the full-effect of these receptors with respect to cellular activation and stability of receptor expression on the cell surface has yet to be determined. Similarly the CAR endodomain has been altered to express a co-stimulatory signaling molecule, such as chimeric CD28, to provide a coordinated signal with CD3- ζ to provide a more complete T-cell activation signal than achieved by signaling through CD3- ζ alone. Because different types of co-stimulation may result in different patterns of cellular activation, it will likely be beneficial to explore alternative co-stimulatory pathways in CAR-mediated T-cell activation. Increasingly, investigators are developing the tools to genetically modify T cells using techniques that cause minimal manipulation of the product leaving intact the full range of T-cell homing and proliferation potentials. Finally, genetic modification is being used to accomplish more than redirect T-cell specificity. For example, experiments are underway to render the T cells resistant to the anti-inflammatory and deleterious effects of iatrogenic glucocorticoids and TGF- β secreted by tumor cell.

Conclusion

Although limitations remain, genetic modifications enable investigators to engineer T cells with augmented

therapeutic potential. It is expected that infusion of genetically modified T cells will be a center-piece of personalized medicine rivaling the influence of therapeutic mAbs as a treatment for malignancies.

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Chimeric Genes

► Fusion Genes

Chimeric Oncogenes

► Fusion Genes

Chimeric Oncoproteins

Definition

Chimeric proteins generated by ► chromosome translocations of two cellular genes in which the functional domains of two separate genes are fused together and/or alter regulation of gene expression. Many chimeric oncoproteins characterized to date appear to act as aberrant transcription factors, likely functioning in
Miltentyi Ex. 1010 Page 7