Designer lymphocytes to fight cancer: a helping hand from modern molecular biology

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Recent advances in molecular biology have spurred a number of exciting developments in cancer therapy. Medical treatment of cancer nowadays is worlds apart from what it used to be even 20 years ago. Cancer therapy has progressively evolved away from the “blunderbuss approach” of old which depended on chemotherapeutic agents and radiation that poorly discriminated between malignant and nonmalignant cells. Today’s “targeted therapy” approaches are significantly more precise and selective and typically exhibit lower toxicity than the older generation of anticancer agents. Small molecule inhibitors block biochemical pathways critical for survival and proliferation of tumor cells. The realization that the immune system can effectively impede cancer progression and even eliminate tumor cells has led to the development of two fundamental strategies for immunotherapy of cancer. Therapeutic antibodies like trastuzumab, rituximab, and alemtuzumab, used independently or in combination with conventional chemotherapy agents, have dramatically improved remission rates and survival in a variety of different cancer types.

The second approach to immunological therapy is based on eliciting a therapeutic immune response against tumor antigens and destruction of the tumor by immune effector cells. Cancer vaccines are based on activating the adaptive immune system in the patients to recognize and eliminate tumor cells. After decades of preclinical studies and clinical trials the first therapeutic cancer vaccine received FDA approval in April 29, 2010. Sipuleucel-T or Provenge cancer

vaccine produced by Dendreon Corporation for use against hormone resistant prostate cancer was recently heralded as triumph for immunotherapy of cancer [1]. The area of cancer vaccine development has advanced painfully slow, much due to the inherent difficulty in evoking an immune response to tumor antigens which often constitute weak “self antigens” expressed in low levels also normal tissues. These difficulties are the results of ingenious mechanisms our immune system has developed to prevent immune response to “self antigens” and avoid “horror autotoxicus” or self-harm, postulated by Ehrlich (1854–1915). These mechanisms incapacitate or attenuate the most important soldiers in the army of immune lymphocyte defenders against cancer, the cytotoxic T cells (CTLs). CTLs optimally should be able to destroy the tumor cells through the injection of pore forming toxins which also trick the tumor cells to commit suicide in much the same way as they can kill virus infected cells. Unfortunately this CTL-mediated destruction of cancer cells, so much sought after by us working in the field of immunotherapy, rarely occurs or is very inefficient at best. T cells which have receptors that bind self-antigens very tightly are eliminated in the thymus through a process coined “negative selection.” Some T cells however manage to sneak through negative selection and migrate into the periphery. Although these T cells generally have receptors which bind inefficiently to their target antigen, they nevertheless can cause havoc and “self-harm,” i.e., autoimmunity. Where it not for powerful mechanisms, such as the regulatory T cells endowed with the capacity to downregulate the activity of self-reactive T cells, the organism would suffer from fatal autoimmune disease, such is the case in mice or patients with genetic defects in their T reg functions [2, 3]. Paradoxically, immune therapy of cancer aims to manipulate the cancer patient’s immune system to a kind of benevolent “horror autotoxicus,” i.e., an experimental autoimmune condition

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specific to the tumor whereby the malignant cells over-expressing certain self-antigens are eliminated while normal tissues only expressing low levels of the same antigens are spared [4].

In addition to the intrinsic difficulties of eliciting a response to weakly immunogenic tumor antigens and the wily ways of the tumor by which they evade immune-mediated destruction, there is one overshadowing reason why cancer vaccines have limited efficacy. T cell clones reactive to a particular antigen are limited in number and even the most effective immunization schedule can expand the reactive T cells only within bounds of homeostasis. As a typical example, keyhole limpet hemocyanin (KLH), a strong xenoantigen, has a precursor frequency of 1:150,000 to 1:340,000 before immunization and reach 1:25,000 to 1:42,000 in individuals repeatedly immunized with KLH [5]. It may be reasonably speculated that weakly antigenic tumor-associated antigens typically elicit fewer T cells compared to KLH following immunization. It therefore becomes rapidly apparent that under the best of circumstances, cancer vaccines almost invariably fall short of eliciting sufficient numbers of tumor-specific T cells required for elimination of a bulky tumor. One attractive way of circumventing the limitations of *in vivo* vaccination is by engineering T cells *ex vivo*, so that they acquire the antigen reactivity of interest. Infusion of these engineered T cells can potentially overcome any limit to the therapeutic efficacy due to restricted numbers of tumor-reactive T cells.

Meyerhuber and collaborators [6] have capitalized on several recent advances in molecular biology and immunology to target the epidermal growth factor (HER) family with T cell receptor gene-modified T lymphocytes. Her2/neu (HER2), the principal tumor target in this study, is expressed on a fraction of nearly all carcinomas as well as on B-ALL. HER2 has already been exploited widely as a target for a number of novel strategies to treat HER2-overexpressing malignancies. Among these, the monoclonal antibody Herceptin has been remarkably successful against breast cancer and is now also being investigated in clinical trials in other malignancies [7]. Also HER2-based tumor vaccines are being tested in clinical trials, but although HER2 specific T cell responses were shown to be induced by these approaches [8], there is still a need for a large controlled HER2 vaccine trial demonstrating clinical efficacy. Since HER2 is a self-antigen expressed at low levels also on normal epithelial surfaces, HER2-specific T cells are being kept in tight control by regulatory mechanisms in the periphery and their T cell receptors (TCR) bind poorly to their targets for reasons discussed above.

Meyerhuber and colleagues have utilized several ingenious measures when developing a novel method to target HER2-overexpressing tumors. Their “adoptive T cell transfer” approach aims to activate and manipulate the patients’ T cells *ex vivo*, without being impeded by mechanisms which dampen T cell responses against self-antigens *in vivo*. The T cells are then transferred back to the

Fig. 1 Schematic representation of the approach to generate TCR-engineered “designer” T cells. T cells of an HLA A2-negative donor is stimulated with HLA A2-positive dendritic cells pulsed with HER2-derived peptides. T cell receptors are isolated from the resulting “alloreactive” T cell clones that demonstrate HLA-A2-restricted, HER2-specific reactivity. The receptor affinity of the TCRs is further improved through codon optimization. T cells from cancer patients that have been expanded *ex vivo* after the introduction of the recombinant TCRs can be used as a homogeneous population of highly reactive immune effectors for adoptive immunotherapy. The author is grateful to Peter Meyerhuber for designing the scheme

patient where they exert antitumor activity. This type of cell therapy is expensive and labor intensive, requiring facilities certified for Good Manufacturing Practice production. Their present study, however, was encouraged by their previous observation that adoptive transfer of HER2-specific CTLs could eliminate disseminated tumor cells in a patient with HER2 expressing breast cancer [9].

Even though this case report was promising, isolation of HER2 specific CTLs in sufficient numbers and of sufficient quality to allow clinically effective, adoptive transfer protocols is virtually impossible for a large group of patients. The investigators therefore added several other sophisticated and innovative modifications to their method. In contrast to weak and inefficient responses that self-antigens evoke, T cell responses to tissue antigens such as those which occur during rejection of transplants between genetically non-identical individuals are extremely powerful. This strong T cell response is directed against non-self human leukocyte antigen (HLA) and is termed an allo-response. It is now generally agreed that during an allo-response between HLA mismatched individuals, CTLs which are specific for peptides bound to HLA molecules are generated as well. Therefore, Meyerhuber and colleagues capitalized on this knowledge and generated a panel of CTLs specific for an immune-dominant HER2 peptide (HER2 369–377) by allo-stimulating T cells from an individual lacking the HLA-A2 antigen with HER2 369–377 peptide pulsed antigen-presenting cells from an individual expressing the HLA-A2 antigen (Fig. 1). Importantly, they had previously shown that these CTLs efficiently could not only recognize HER2 specific HLA A2+ tumors but could also cross react with the analogous nonamer HER2 epitope derived from the HER3 and HER4 members of the EGF receptor family [10].

The cumbersome task of isolating large numbers of tumor-specific T cells for adoptive T cell therapy can be circumvented by transferring the patients' own T cells back after modifying them *ex vivo* to express TCR derived from a clone of tumor specific T cells. Thus, a large number of the patients' own T cells can be genetically manipulated to express the same TCR. This approach potentially facilitates individualized tumor therapy with tailor made TCR reactive to the specific tumor antigen expressed on the patients' tumor. Clinical trials are already ongoing demonstrating the feasibility and occasional tumor regressions of this approach [11]. With this strategy in mind, Meyerhuber and colleagues isolated the TCR genes of their HER2-reactive allo-HLA-A2-restricted CTL and introduced them into a retrovirus vector (Fig. 1). Since the cell-surface expression of gene-transferred TCRs is often poor, they optimized TCR transgene expression by measures such as codon optimization and replacing the human TCR constant regions with the mouse counterparts. In a series of

experiment, they demonstrated that human T cells transduced with this optimized HER2-TCR not only showed similar antigen recognition as the parent CTL clone, but that the transduced T cells retained their ability to efficiently recognize tumor cells expressing HER2 as well as the other EGF family members HER3 and 4.

The clinical implications of this tour-de-force, combining recent developments in molecular biology with T cell immunology, are enormous. The prospect of treating solid tumors by simultaneously targeting HER2 as well as HER3 and HER4 is very attractive since it mitigates the probability of escape variants that have downregulated HER2. Nevertheless, the possibility of severe adverse events associated with the transfer of a large number of lymphocytes is very real. Morgan et al. administered a large number of T cells transduced retrovirally with a chimeric antigen receptor based on the monoclonal antibody Herceptin to a patient with HER2 expressing colorectal cancer [12]. This treatment proved fatal for the patient which possibly resulted from the large number of administered cells localizing to the lungs and the ensuing "cytokine storm" following recognition of low levels of HER2 on lung epithelial cells. However, great advances usually occur with bold initiatives and with extensive preclinical testing. Meyerhuber and colleagues can potentially make inroads into transforming Erlich's "horror autotoxicus" into tomorrow's immunotherapy against cancer.

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