Chapter 1

Introduction to Engineering Immunity

1. Immunotherapy

The goal of engineering immunity is to harness and engineer the immune system in order to treat infectious diseases or cancer. The concept of immunotherapy has been successfully used to prevent and fight many deadly diseases. Immunotherapy essentially started with the revolutionary invention of vaccination more than 300 years ago, when Edward Jenner discovered that deliberate infection with attenuated cowpox virus induces mild disease, and the resultant immunity can circumvent smallpox infection (Jenner, 1798). Table 1-1 lists milestone events in the history of immunotherapy.

It was found that a similar vaccination approach is especially difficult to treat some chronic infectious diseases such as human immunodeficiency virus (HIV) or cancer, largely due to negative immunoregulatory mechanisms (Waldmann, 2003). Recent advances in understanding the nature of immune response, immunological signaling pathways, and immunological mediators has led to several new promising therapeutic strategies, which are briefly highlighted here with a focus on cancer immunotherapy.

New Generation Vaccines:

Given the vital need for effective therapies for cancer and for certain chronic diseases, the development of a new generation of vaccines has been at the forefront of active immunotherapy research. With these diseases, live attenuated pathogens are not necessarily optimal to induce an appropriate magnitude of immune response. In the case of cancer, self-tolerance

mechanisms could delete most responsive T cells against antigenic epitopes of tumor cells. In order to generate a more immunogenic response, epitope enhancement has been used by alternating the sequence of antigens (Berzofsky, 1993). One approach that uses this method is to select the high affinity of the epitope peptide for a major histocompatibility complex (MHC) molecule. Several peptides have been identified with enhanced binding potential towards MHC for treatment of HIV and cancer (Ahlers et al., 1997; Irvine et al., 1999; Parkhurst et al., 1996; Pogue et al., 1995; Rosenberg et al., 1998; Sarobe et al., 1998). One could also modify the epitope sequence to elevate the binding affinity of peptide-MHC complex for the T cell receptor, resulting in the more effective T cell immunity. This is especially applicable to tumor antigen epitopes because self-tolerance mechanism leaves only low avidity T cells. Several altered peptide ligands showed the elevated capacity to stimulate and expand reactive T cells in vivo (Fong et al., 2001; Slansky et al., 2000; Zaremba et al., 1997).

Reformulation of vaccines by using combinations of all types of adjuvants, including multiple peptides or proteins, antigen-loaded dendritic cells, recombinant viruses or bacteria and naked DNA can be also effective to elicit appropriate magnitude of immune response. Adjuvants are important components of most cancer vaccines, and could facilitate the activation of antigen presenting cells (APCs) to stimulate T cells more efficiently. In principle, any molecules capable of boosting immune response can be included in adjuvant agents. Cytokines, such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), IL-12, IL-4, etc, have been added as adjuvant in

cancer vaccines (Salgaller and Lodge, 1998). Lipopolysaccharide (LPS) and monophosphoryl lipid A (MPL) produced by bacteria are known to be effective in activating cytotoxic T lymphocytes (CTLs). Since bacterial DNA contains unmethylated CpG dinucleotides, it has been found recently to be an efficient immunostimulatory molecule (Krug et al., 2003; Medzhitov, 2001). Given that antigen ends up in cytoplasm for further processing and presentation, it is beneficial if one can develop adjuvant capable of delivering antigen to cytoplasm. Microparticles (Lo-Man et al., 1998; O'Hagan et al., 1998) and immunostimulatory complexs (ISCOMs) (Takahashi et al., 1990) have been reported to be able to efficiently deliver antigen to the cytoplasm. Heat-shock proteins can also facilitate the intracellular delivery of antigens to APCs and promote the presentation to MHC molecules (Srivastava, 2002).

Dendritic cell (DC) based vaccines shows a great promise for cancer vaccine development (Nestle et al., 2001). Tumors (Celluzzi and Falo, 1997; Celluzzi and Falo, 1998), apoptotic bodies (Nouri-Shirazi et al., 2000), tumor lysates (Chang et al., 2002), tumor RNA (Heiser et al., 2001) and tumor DNA (Condon et al., 1996; Whiteside et al., 2002) can be loaded to DCs. Most of these approaches showed great success in tumor rejection in animal models and many of them are in different phases of human clinical trials.

Target the Negative Immunoregulatory Mechanism:

In order to prevent self-destructive immune response that could cause autoimmune disease, the immune system has evolved a range of negative

immunoregulatory mechanisms to ensure safety. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a negative co-stimulatory molecule; its expression is usually induced upon T cell activation (Chambers et al., 2001). As a binding competitor, higher affinity of CTLA-4 toward B7 can block the co-stimulatory interaction of CD28 with B7, which is vital for T cell activation. Allison and co-workers demonstrated that antibody blocking CTLA-4 action can improve T cell response against tumors and the regression of established transplanted tumors was observed (Chambers et al., 2001). Inhibition by regulatory T cells provides another means of negative control for the maintenance of self-tolerance. Among CD4 T cells, there is a population of $CD4^{+}CD25^{+}$ T cells that impedes T cell action against tumor antigens. Administration of monoclonal antibody directed to regulator T cells in mice showed decreased numbers of circulating $CD4^{+}CD25^{+}$ T cells, resulting in the rejection of certain syngeneic tumors (Sakaguchi et al., 2001; Sutmuller et al., 2001).

Monoclonal Antibodies for Passive Immunotherapy:

In 1900, Paul Ehrlich implied that molecules that react with tumors could be useful for cancer therapy, suggesting a possible approach of antibody-mediated passive immunotherapy (Ehrlich, 1956). In the last decade, significant advances have been made toward the use of monoclonal antibodies against cancer cells. The two most successful antibodies are rituximab (Rituxan) and trastuzumab (Herceptin). Patients with non-Hodgkin lymphoma exhibited an overall response rate of 57% after receiving rituximab treatment (McLaughlin,

1999; McLaughlin et al., 1998a; McLaughlin et al., 1998b; McLaughlin et al., 1998c). Transtuzumab is a humanized antibody against the tyrosine kinase receptor HER2/neu and administration of this antibody achieved an overall response rate of 15% of patients with breast cancer expressing the high level of HER2 (Baselga et al., 1998; Baselga et al., 1999).

In order to increase the therapeutic efficacy of antibodies, several strategies of modifying and redesigning antibodies have been developed. Clynes and co-workers observed that interaction of antibodies with Fc-γ receptors on the surface of effector cells is the major contribution in eliciting antibody-dependent cellular cytotoxicity (ADCC) (Clynes et al., 2000; Ravetch and Clynes, 1998). Furthermore, they found that engagement of Fc-γ RIII to antibodies can promote ADCC, while antibodies binding to Fc-γ RII can inhibit ADCC. Thus engineering antibodies with high affinity toward Fc-γ RIII and low affinity toward Fc-γ RII can increase their therapeutic impact. Antibodies conjugated with toxins and radionuclides can increase their cytotoxicity and make them better cytocidal agents (Waldmann, 2003). A CD33-specific antibody armed with immunotoxin calicheamicin has been approved by FDA to treat myelogenous leukemia (Kreitman et al., 1998). Compared to unmodified antibody, radiolabeled CD20-specific antibody showed enhanced efficacy for treatment of non-Hodgkin lymphoma (Witzig et al., 2002).

2. Cancer T Cell Therapy

As we mentioned, the feasibility of using monoclonal antibodies against cancer has been firmly validated by recent clinical success in breast cancer and B cell lymphomas. T cell based immunotherapy in principle should offer even wider therapeutic potential because of the nature of T cell immunity (Pardoll, 2002a). Significant advances have been made in elucidating of the antigenic mechanism of tumor recognition and destruction by T cells. It becomes clear that cytotoxic T lymphocytes (CTLs), or $CD8^+$ T cells, are the effector T cells to ultimately take the action to reject tumor cells (Dudley and Rosenberg, 2003). CTLs cause the destruction of tumor cells by several possible mechanisms, including the release of inflammatory cytokines such as tumor-necrosis factor α (TNF-α) and interferon γ (IFN-γ), expression of apoptosis-inducing surface ligands such as FAS ligand (FASL) and TNF-related apoptosis-inducing ligand (TRAIL), and cytotoxic degranulation, which triggers the perforin-mediated lysis (Dudley and Rosenberg, 2003; Guidotti and Chisari, 2001; Harty et al., 2000). However, much evidence gleaned from clinical practices and animal models showed that circulating tumor-specific CTLs are not sufficient to reject the established tumors. An appropriate vaccination method can readily generate tumor-reactive CD8 T cells *in vivo*, but vaccination alone rarely causes complete regression of tumors. Even in transgenic models, in which every CD8 T cell expresses tumor-specific T cell receptors (TCRs), tumors are still able to grow (Dudley and Rosenberg, 2003; Prevost-Blondel et al., 1998; Wick et al., 1997).

The exact reason that causes the failure of complete tumor elimination after vaccination or in TCR transgenic animals is not completely understood.

Many mechanisms have been proposed to account for such a failure (Table 1-2, (Dudley and Rosenberg, 2003)): many experiments showed that most tumor reactive CD8 T cells isolated from tumor sites have attenuated function, which is called anergy (Staveley-O'Carroll et al., 1998); T cell differentiation was proven to be difficult in the tumor microenvironment (Ochsenbein, 2002; Ochsenbein et al., 2001); the negative regulatory mechanism provided by $CD25^{+}CD4^{+}$ can impede the immune reaction against tumors (McHugh and Shevach, 2002; Shevach, 2002; Shevach, 2003; Shimizu et al., 1999); as tumors progressively grow, additional suppression mechanisms such as down-regulation of TCR and MHC expression could occur (Dudley and Rosenberg, 2003); tumors can evolve to lose or mutate tumor-antigens to escape TCR recognition (Khong and Restifo, 2002); etc.

Facing such difficult situations, recently many exciting developments on T cell based cancer immunotherapy have been achieved and many new strategies have been demonstrated. In particular, recent two clinical studies using adoptive transfer of tumor-specific T cells demonstrated that *in vitro* activated T cells can inhibit tumor growth. These trials provide many new insights and hopes for T cell immunotherapy (Dudley et al., 2002; Yee et al., 2002).

Adoptive Immunotherapy:

T cell adoptive immunotherapy involves the selection of tumor reactive T cells with optimal characteristics such as expression of TCRs with high avidity against tumor antigens. The selected T cells are then expanded in vitro with the

suitable stimulation condition. The expanded cells are transferred back to the host; this step may require the pre-treatment of the host with immunosuppressive drugs to ensure that the host environment can adapt the transferred cells into functional T cells. As shown in Figure 1-1, this entire procedure provides a number of opportunities to circumvent the inefficient immune response generated by the tumor-bearing host (Dudley and Rosenberg, 2003; Ho et al., 2003; Pardoll, 2002b). For example, the selection step could yield T cells with high reactivity toward tumor antigens, which are normally deleted from T cell repertoire in host owing to self-tolerance mechanisms; the in vitro expansion step could render a large number of tumor reactive T cells; immune suppression of host could create a favorable environment for the transferred highly tumor-reactive T cells to be maintained.

After several failures of pre-clinical trials, Rosenberg and co-workers conducted a new trial with an improved protocol (Dudley et al., 2002). Tumor-infiltrating lymphocytes (TILs) were isolated from 13 patients with metastatic melanoma and cultured in vitro. The digested tumor tissue was added as antigen-loaded APCs and large quantities of IL-2 were supplied into the cultured medium to promote T cell proliferation. Highly tumor-reactive T cells were selected by cytokine secretion assay and further expanded in medium containing high concentration of IL-2. Once over $5x10^7$ T cells from TIL cultures with specific tumor recognition were obtained, a rapid expansion protocol was employed to achieve roughly 1000-fold increase of the desired T cells. All the patients had a prior lymphodepletion before receiving adoptive transfer of

approximately $8x10^{10}$ in vitro-expanded cells. Results from clinic were very impressive: 6 of the 13 patients had true partial responses to treatment (50% reduction at all tumor sites) and an additional 4 of 13 patients exhibited mixed responses (defined as shrinkage of some lesions but growth at others). In addition to tumor regression, some pronounced immunological findings enhance the potency of this approach: in vivo proliferation of the transferred cells was observed in some patients; two patients developed stable engraftment of single T cell clones (60% of the total peripheral blood CD8 T cells in one patient and 75% of the CD8 T cells in another patient).

In parallel studies, Greenberg and co-workers performed a similar trial with a different protocol (Yee et al., 2002). Peripheral blood lymphocytes (PBLs) were grown in medium supplemented with antigen-loaded dendritic cells, the most powerful stimulator for T cells. The tumor antigens employed in the trial were two well-characterized melanoma antigens: MART-1 and gp100. After 3 times of weekly stimulation, most of the T cells were unable to elicit the proper anti-tumor responses. A chromium release assay was used to select clones capable of lysing antigen-bearing tumor cells. Greenberg and co-workers were able to identify such T cell clones, indicating existence of tumor reactive T cells in host, which can be activated and expanded. These T cell clones were then rapidly expanded with the addition of anti-CD3 antibody, irradiated allogeneic PBMCs, irradiated allogeneic lymphoblastoid cell line and IL-2, in medium. Approximately 6 billion of such T cells with pure antigen specificity were transferred back to patients every 2 weeks. For the first infusion of T cells, no IL-

2 was administrated. At the subsequent infusion, low dosage of IL-2 was supplied to patients, with the consideration that low amount of IL-2 could selectively activate T cell clones with expression of high affinity of IL-2 receptors. Analysis of the 10 patients receiving such T cell infusion provided many important insights on adoptive T cell transfer. FACS staining using antigen-MHC tetramer for patients' peripheral blood clearly showed the persistence of the transferred T cells. The half-life for T cells without administration of IL-2 was about 1 week and was extended to 2.5 weeks when IL-2 was used, indicating that IL-2 can prolong the life of antigen-specific cytotoxic T lymphocytes. Analysis of tumor biopsies from patients also revealed that 37% of tumor infiltrated T cells were tumor-antigen positive and less than 1% of these T cells resided in peripheral blood, suggesting that the infused antitumor T cells indeed were able to traffic to tumor sites. Unfortunately, none of the patients showed objective clinical responses to the treatment (shrinkage of tumor more than 50%). Examination of tumors from patients revealed the loss of the melanosomal antigen (either MART-1 or gp100) in 3 out of 5 patients, confirming the genetic instability of tumor cells.

Nevertheless, Greenberg's trial demonstrated that adoptive transferred T cells could persist in host and migrate to tumor sites. Coupled with successful example of Rosenberg's trial and other previous reports, these series of experiments show promise of adoptive T cell therapy in treatment of several diseases, including CMV, EBV, melanoma and leukemia (Dudley et al., 2002; Rooney et al., 1995; Walter et al., 1995; Warren et al., 1998; Yee et al., 2002).

With current pace of progress in understanding the lymphocyte characteristics and the role of the host immune environment on the efficacy of adoptive therapy, this therapeutic method will become a reliable clinical practice in the near future.

Redirecting T Cell Specificity:

As we described previously, CTLs are required to efficiently eliminate tumors. Both Rosenberg and Greenberg chose melanoma for T cell-based immunotherapy in part because it is relatively easier to grow tumor-specific CD8 T cells from melanoma patients than any other types of cancer. Generation of active, tumor-specific lymphocytes is still a very difficult task (Ho et al., 2003). Alternatively, one can redirect T cell specificity against tumor antigens by TCR gene transfer (Pardoll, 2002b; Sadelain et al., 2003). As shown in Figure 1-2, this approach may seem to be very straightforward, but it has been proven to be quite difficult, presumably due to the nature of TCRs. Fortunately, much progress has been made on this front, and several groups have reported the successful transfer of TCR gene into T cells, with the resulting T cells exhibiting the desired antigen specificity (Clay et al., 1999a; Clay et al., 1999b; Cooper et al., 2000; Fujio et al., 2000; Kessels et al., 2000; Kessels et al., 2001; Stanislawski et al., 2001).

Nishimura and co-workers reported the first example of genetic modification of human peripheral blood (PBL) by virus-mediated transfer of TCR genes (Clay et al., 1999b). Genes encoding α and β chains of TCR against melanoma associated antigen MART-1 were cloned into A7 retrovirus.

Expression of α chain was driven by 5' LTR promoter, and β chain expression was under control of the hybrid SRα promoter. After virus transduction, over 22% of PBL cells were able to express a MART-1 specific TCR. Clones isolated from these transduced cells could respond to antigenic stimulation and secrete IFN-γ, demonstrating the idea of redirecting antigen specificity of T cells.

Since then, several other groups also reported the use of TCR gene transfer to edit T cells (Cooper et al., 2000; Fujio et al., 2000; Kessels et al., 2000; Kessels et al., 2001; Stanislawski et al., 2001). Two recent impressive papers from the Theobald group (Stanislawski et al., 2001) and the Schumacher group (Kessels et al., 2001) showed the success of retroviral delivery of TCR genes into T cells, and demonstrated that the transduced T cells are functional in vivo. Schumacher and co-workers inserted the cDNA of F5 TCR recognizing the H-2D^b-restricted, influenza derived NP antigen, into a moloney-based retroviral vector (Kessels et al., 2001). Retrovirus-mediated virus infection resulted in up to 20% of the T cells expressing functional F5 TCR. These cells were adoptively transferred into animals. Up to 1000-fold expansion of transferred T cells was observed after influenza infection. They further demonstrated that immuno-compromised RAG-deficient mice, after receiving the redirected T cells, could survive from tumor challenge.

Theobald and co-workers took advantage of the approach of redirecting T cell specificity to overcome tumor immune responses imposed by immunological tolerance (Stanislawski et al., 2001). A peptide, designated as MDM2, a potential antigen for many malignant cells, was isolated from the HLA-A2

complex and found to be able to induce high magnitude immune responses using T cells derived from individuals lacking HLA-A2 expression. In contrast, MDM2 could not elicit even modest immune reactions in human expressing HLA-A2; this outcome may be due to low presentation of such an antigen on MHC so that the cognate T cells are deleted during positive selection. After transfer of murine derived, humanized MDM2 responsive TCR gene into HLA-A2 positive human T cells, the resultant T cells were able to kill tumors bearing MDM2 antigen.

The ability to redirect T cell specificity by TCR gene transfer expands the scope of adoptive transfer when the suitable antigenic T cell clones are unable to be isolated from patients. This method also engenders another exciting opportunity for building better T cells. One obstacle for the success of adoptive therapy is the lack of availability of sufficiently high-avidity tumor reactive T cells (Dudley and Rosenberg, 2003; Ho et al., 2003). TCR gene transfer may help overcome this problem. With TCR gene transfer, one can clone the low affinity TCR into a retrovirus vector. A library of TCR can be generated by PCR mutation. The library can then be displayed on the surface of yeast (Holler et al., 2000) or on retrovirually transduced T cells (Kessels et al., 2000). Appropriate screening methods such as peptide-MHC tetramer staining can be applied to select for high affinity TCR (Holler et al., 2000). However, whether the in vitro-generated high affinity TCR could be applicable in vivo remains unclear. One obvious concern is the possibility of breaking the immunological tolerance. Also the engineered T cells may have the reduced ability to migrate so that they may essentially have impaired effector function.

TCR gene transfer to redirect T cell specificity has great potential in immunotherapy. It provides an alternative way to generate a large quantity of T cells with anti-tumor specificity in vitro and creates new prospects for the research of T cell biology, tumor immunity and cancer T cell therapy.

Chimeric Antigen Receptors:

T cell therapy using either natural occurring αβ TCRs or transduced TCRs has a few of potential limitations. TCR-mediated antigen recognition requires the recognition of MHC on antigen presenting cells. For tumor therapy, one has to isolate a range of T cell clones that recognize the same tumor antigen but with different MHC restrictions. This is a very demanding task and certainly complicates the clinical application of this method. Another limitation imposed by TCR-based T cell therapy is that only protein-based tumor antigens can be targeted; carbohydrate and glycolipid are two important categories of non-protein tumor antigens (Sadelain et al., 2003).

There is another class of investigation on genetic modification of T cells with a strategy of generating tumor-specific T cells using gene transfer of chimeric antigen receptors (CARs, Figure 1-3). CARs are composed of an antigen recognition domain fused to a cytoplasmic portion of T cell activation domain. On a natural T cell surface, the T cell uses two different molecules to perform recognition and activation: antigen recognition is realized through the α and β chains of TCR, while the activation signal is mediated through the CD3 complex, which consists of a δε-chain heterodimer, a γε-chain heterodimer and a

ζ-chain homodimer (Figure 1-4A) (Germain and Stefanova, 1999). Binding of the TCR to a peptide-MHC complex on an APC surface can induce cross-linking of the TCR and trigger the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs), which are parts of CD3 complex, initiating the activation of T cell (Germain and Stefanova, 1999). CAR combines these two functions in a single molecule (Figure 1-4B). The recognition domain of CAR can be derived from a monoclonal antibody, receptor or surface ligand. And the activation domain can be signaling motifs of CD3 complex. It is hoped that upon binding of antigen, the CAR will be cross-linked and activation motifs can then be phosphorylated in the same way as in physiological TCR signaling.

Eshhar and co-workers reported their pioneer work on CAR in 1993 (Eshhar et al., 1993). They constructed a CAR, which included a hapten-specific, immunoglobulin-derived single-chain variable fragment (svFv) as the recognition domain, and a cytoplamic region of the CD3ζ chain. This chimeric receptor gene was transferred into a mouse CTL hybridoma cell line and the resultant cells were able to respond to hapten-bearing cells. In addition, they showed that the activation motif derived from the FcεRγ chain, which mediates activation signal of the receptor FcεR1 expressed on the surface of natural-killer cells, could also serve as an activation motif for the CAR (Figure 1-4B). In the subsequent paper, the same group constructed another CAR in mouse CTL hybridoma to target tumor cells expressing the HER2/neu (Eshhar et al., 1993).

Over the last decade, many cancer antigens have been targeted by the engineered CARs (Abken et al., 2003; Altenschmidt et al., 1997; Beecham et al.,

2000a; Beecham et al., 2000b; Chames et al., 2002; Daly et al., 2000; Darcy et al., 2000; Haynes et al., 2001; Haynes et al., 2002; Hombach et al., 2001; Hombach et al., 2000; Hwu et al., 1995; Kershaw et al., 2000; Krause et al., 1998; Maher et al., 2002; McGuinness et al., 1999; Niederman et al., 2002; Nolan et al., 1999; Parker et al., 2000; Patel et al., 2000; Ren-Heidenreich et al., 2002; Sadelain et al., 2003; Wang et al., 1998; Yun et al., 2000). These experiments have not only demonstrated that CARs can direct the hybridoma T cell lines to the intended tumor antigens, but also confirmed that the primary T cells either from murine or human, once transduced with CARs, can convey anti-tumor specificity. In animal models, CARs have been used in vivo efficiently to target tumor antigens ERBB2 (Altenschmidt et al., 1997), the colorectal carcinoembryonic antigen (CEA) (Darcy et al., 2000; Haynes et al., 2002), the adenocarinoma antigen TAG-72 (McGuinness et al., 1999), and ovarian carcinoma antigen FBP (Hwu et al., 1995; Wang et al., 1998). Sadelain and co-workers recently showed that human PBLs, infected with the designed CARs, after adoptively transfer into SCID mice, were able to reject established lymphomas. The investigators also demonstrated that expansion in the presence of IL-15 was vital for persistence of adoptively CAR-transferred T cells and the therapeutic efficacy (Brentjens et al., 2002).

With no MHC restriction, the approach using CAR-modified T cells offers significant advantages over the physiological T cell approach on adoptive T cell therapy. However, this method contains certain limitations. The biggest limitation is the quality of activation signal elicited by CARs. This is in part

manifested by a modest in vivo anti-tumor response when CAR-modified T cells were adoptively transferred into tumor-challenged mice. To address this limitation, Hwu and co-workers imparted dual specificities into targeted T cells to enhance the anti-tumor activity (Kershaw et al., 2002). In their experimental procedure, the CAR-directed T cells were first activated via their physiological TCRs stimulated by the allogeneic stimulator cells. This subset of T cells, once expanded, conferred dual specificities: one is provided by CARs and anther is provided by natural allogeneic responsive TCRs. These selectively expanded cells were then transferred back to the tumor-bearing host mice. Immunization by allogeneic cells could further expand and activate the transferred T cells. Anti-tumor specificity conveyed by CARs directed these cells into tumor sites. Since these cells had been activated by their natural TCRs, they had more potential to kill tumor cells. As expected, they observed more potent ability for these dual specific T cells to eliminate tumors.

3. The Unique Role of CD4 T Cells in Anti-tumor Immunity

As we mentioned before, the role of CD8 T cells in anti-tumor immunity is is to perform tumor killing upon direct recognition of tumor antigens presented by MHC I. What roles CD4 T cells play in the tumor battle, however, needs further investigation.

There is accumulating evidence showing that CD4 T cells have an indispensable role in antitumor immunity. Several tumor antigens recognized by CD4 T cells in the context of MHC II molecules have been identified from tumor

patients (Renkvist et al., 2001). Direct evidence for the critical role of CD4 T cells in induced tumor immunity comes from experiments with depletion of CD4 T cells by antibody blocking or in CD4 knockout animal models (Cavallo et al., 1992; Dranoff et al., 1993; Golumbek et al., 1991; Hock et al., 1991; Levitsky et al., 1994; Ostrandrosenberg, 1994; Pan et al., 1995; Pulaski et al., 1993). Several lines of evidence consistently demonstrated that depletion or absence of CD4 T cells attenuated significantly the ability of the immune system to reject tumors. In the experiment for evaluating separate contributions of T cells in the elimination of the Friend murine leukemia virus (MuLV)-induced tumor FBL-3, the investigators found that CD4 T cells can elicit the antitumor immunity independent of CD8 T cells (Greenberg, 1991; Greenberg et al., 1985). One of Greenberg's experiments showed that adoptive transfer of CD4 T cells into mice bearing MHC II-negative tumors resulted in the control of tumor growth via mechanisms requiring no CD8 participation (Greenberg et al., 1985). Tumor vaccination experiments revealed that simultaneous vaccination with the tumor-related CD4 epitope and CD8 eptitope resulted in much better tumor responses than with a tumor-unrelated CD4 epitope (Ossendorp et al., 1998). Further evidence can be derived from tumor-specific CD4 T cell tolerance. Levitsky and co-workers adoptively transferred transgenic CD4 T cells with specificity against tumors and observed progressive induction of tumor-specific anergy of these cells after tumor challenge (Staveley-O'Carroll et al., 1998). Blankenstein and co-workers reported that antitumor responses exhibited a surprising increase in mice lacking B cells, and argued that presentation of tumor antigens to B cells

could redirect the CD4 reaction into the non-productive form of humoral immune (Th2 type) response instead of the productive (Th1-type) response (Qin et al., 1998). Pardoll and co-workers suggested that the degree of unresponsiveness of CD4 T cells against tumors is more pronounced, as evidenced by the cells encountering anergy induction or clonal diversion, while tolerance induction of CD8 T cells is more passive, as shown by the cells' ignorance of antigen or failure to trigger memory responses (Pardoll and Topalian, 1998).

Thus, it becomes clear that the involvement of CD4 T cells is critical for generation of productive immunity against cancer. Dissection of the cellular and molecular interactions pertaining to anti-tumor reaction revealed that CD4 T cells orchestrate multiple effector functions, and collaborate with CD8 T cells to yield effective antitumor immune responses. At first, CD4 T cells provide tremendous help to induce CD8 T cell-based antitumor responses. In addition to secreting cytokines, CD4 T cells provide indirect help to CD8 T cells by stimulating APCs (Bennett et al., 1997). Heath and co-workers demonstrated that CD8 T cell priming requires the co-recognition of antigens by both CD4 and CD8 T cells on the same APCs in an MHC restricted manner, which explained the previous finding that cytokines such as IL-2 can only transfer CD4 help to the proximal CD8 T cells (Bennett et al., 1997). It was discovered that interaction between CD40 on the CD4 cell surface and CD40L on APCs is essential to activate APCs to be able to prime cognate CD8 T cells (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998). Blocking the binding of CD40/CD40L led to inefficient priming of CD8 cells and subsequent induction of immune responses

(Koch et al., 1996). CD40 ligation could upregulate the expression of CD80, CD86 and intercellular adhesion molecule 1 (ICAM-1), which are important co-stimulatory molecules for priming CD8 T cells (Cella et al., 1996; Shinde et al., 1996). This evidence agrees with the model that APCs cross-present cognate antigens for both CD8 and CD4 T cells to allow the delivery of CD4 help to proximal CTL CD8 T cells (Figure 1-5).

The accumulating evidence also implicates that CD4 T cells can have several routes to contribute their antitumor immunity independent of CD8 T cells. In an early experiment, Greenberg found that the adoptively transferred tumor-specific CD4 T cells could activate tumoricidal macrophages that were useful for control of tumor growth (Greenberg, 1991). Recent tumor cell-based vaccination experiments revealed that Th1 and Th2 CD4 T cells, in response to vaccines, could release their cognate cytokines, which in turn recruit and activate macrophages and eosinophils, respectively (Hung et al., 1998). Thus, for the CTL independent model, CD4 T cells activated by tumor antigens could mediate the production of oxygen radicals (nitric oxides or superoxides) by macrophages, and the release of granule contents by eosinophils, to empower the antitumor reaction (Figure 1-5).

Appreciation of the pivotal roles of CD4 T cells in generating efficient antitumor immunity is of importance for design of cancer vaccines and T cell therapy to achieve maximal therapeutic efficacy.

4. Hematopoietic Stem Cells As Targets for Gene Therapy

Hematopoietic stem cells (HSCs) are multipotent stem cells that can develop into any types of cells in peripheral blood (Bodine et al., 1998; Brown et al., 2001; Kondo et al., 2003; Orlic et al., 2001; Weissman, 2002; Weissman et al., 2001). Millions of mature blood cells can be generated from HSCs via the extensive proliferation and differentiation in hematopoietic system. In general, HSCs can differentiate into lymphoid (B and T cells), myeloid (granulocytes and monocytes), megakaryocytic (platelets) and erythroid (red cells) lineages (Figure 1-6). One important property of HSCs is that they can self-renew without lineage changes (Eaves et al., 1991; Thomas et al., 1975a; Thomas et al., 1975b). The ability of self-renewal makes HSCs powerful sources for transplantation applications, since a small number of HSCs can repopulate the entire hematopoietic system for patients receiving radio- or chemo-therapy.

Mouse Hematopoietic Stem Cells:

Several experiments have demonstrated that mouse HSCs can repopulate the entire hematopoietic system after transfer into irradiated hosts (Orlic et al., 2001; Weissman et al., 2001). In these animal experiments, it was shown that repopulating hematopoietic system of mice only require a single HSC, although the minimal number for the transferring bone marrow cells has to be larger than $1x10^5$ (Boggs et al., 1982; Harrison et al., 1988; Nakano et al., 1989). Powerful assays have also been developed to evaluate and quantify the relative ability of individual genetically distinguishable HSCs to repopulate recipient mice (Micklem

et al., 1972). These assays estimated that in adult mice, 80% of HSCs are located in the bone marrow, 19% of HSCs reside in the spleen, and only 0.5% of HSCs can be identified in the peripheral blood (Bodine et al., 1995; Bodine et al., 1994). When treating the mice with hematopoietic growth factors or antitumor drugs, investigators also discovered the redistribution of HSCs in the PBL (Bodine et al., 1996; Brasel et al., 1995a; Brasel et al., 1995b; Brasel et al., 1997; Brasel et al., 1996; Molineux et al., 1997; Neben et al., 1993; Neipp et al., 1998). In one example, Granulocyte-Colony Stimulating Factor (G-CSF) was administered to animals and it was observed that 88% of HSCs occupy the spleen and 2% of HSCs reside in the peripheral blood (Bodine et al., 1995; Bodine et al., 1994; Bodine et al., 1996). When mice were treated with cyclophosphamide (CP), an antitumor agent, a 30-fold increase of HSCs in the PBL was found (Neben et al., 1993).

Human Hematopoietic Stem Cells:

Bone marrow transplantation has become a common clinical practice in the treatment of many inherited and acquired hematopoietic diseases. Success of these treatments relies on the fact that human hematopoiesis can be regenerated from a small number of transplanted HSCs. Although bone marrow and the PBL cells are the most available sources of human HSCs, cord blood and fetal liver HSCs can be alternative sources (Amos and Gordon, 1995; Broxmeyer, 1995; Russell et al., 1998). Cord blood as a rich source for HSCs has been exemplified by an experiment showing that HSCs derived from 100 mL

cord blood are adequate to regenerate a person's hematopoietic system (Broxmeyer, 1995).

It is interesting to revisit some of the animal models developed when investigators tried to identify an in vivo transplantation assay for human HSCs. The early successful animals used for human HSC transplant were immunodeficient sheep (Shimizu et al., 1998; Zanjani, 2000; Zanjani et al., 1995a; Zanjani et al., 1996). Usually the rapid expansion of the hematopoietic system of sheep begins at day 50 of gestation, while its immune system is not functional until day 70 of gestation. Thus, between days 50 and 70 of gestation is the best time window to infuse human HSCs. The expansion period can allow human HSCs to expand efficiently and the immune deficiency can allow sheep to develop tolerance toward human antigens. The success rate for such transplantation is about 70% and human cells can occupy the average of 5% of cells in the sheep peripheral blood. The properties of human HSCs such as self-renewal are retained. The persistent human cells in sheep can be expanded by administration of human cytokines such as IL-3, GM-CSF, Stem Cell Factor (SCF), etc (Srour et al., 1992a; Srour et al., 1992b; Srour et al., 1993; Zanjani et al., 1995b).

Other animal models pursued are immunodeficient mice (Dao et al., 1999). To date, human HSCs have been successfully transplanted into BNX mice (beige, nude, xid) and SCID (severe combined immunodeficiency) mice (Dick, 1997; Shultz, 1991). BNX mice are completely immune deficient due to the accumulated three mutations (the *beige* mutation causes natural killer cell

deficiency; the *nude* mutation causes thymus deficiency; the *xid* mutation causes the functional deficiency of B cells). Unlike the transplantation experiments done for sheep, less than 1% of human cells could be identified in BNX mice's bone marrow and spleen (Kamelreid and Dick, 1988). Inspired by the fact that human cytokines such as IL-3 and GM-CSF can facilitate the expansion of human cells, investigators found that infusion of human stromal cells capable of producing human IL-3 can increase the percentage of human cells to 6% in recipient bone marrow (Nolta et al., 1994). When SCID mice were used as recipients to receive human bone marrow transplantation, as much as 3% of human cells can be found in mouse bone marrow (Bock et al., 1993; Bock et al., 1995; Lapidot et al., 1992; Lubin et al., 1991). Shultz and co-workers demonstrated that when SCID strain was crossed into NOD strain (NOD strain mice are susceptible to Non-Obese Diabetes), the resulting NOD/SCID mice were more immunodeficient, and were better than SCID in engrafting human HSCs (Kollet et al., 2000; Shultz et al., 1995).

Recently, notable work has been done by Weissman's group at Stanford University, showing that infant mice, once infused with human cord blood cells, can maintain human cells as the mice grow (Personal Communication). Analysis of the adult mice demonstrated the presence of human B- and T-lymphocytes in the peripheral blood. Class switch upon antibody production in the human B cells was also observed, implying that the generated human CD4 T cells are functional (to mediate B cell class switch). This opens up a new arena for studying human HSC biology and human immunology in mice.

***HSC Gene Therapy*:**

The biological capacities of HSCs are enormous (Kondo et al., 2003). The fact that HSCs can repopulate the host's hematopoietic system makes them particularly desirable to be used for correcting some inherited or acquired diseases (Anderson, 1984; Anderson, 1992; Bordignon and Roncarolo, 2002; Dunbar, 1996; Dunbar, 1998; Hu and Dunbar, 2002; Kohn, 1999). HSCs integrated with new genes can expand the scope and utility of bone marrow transplantation (BMT), because the recipients then have a continuous stream of intended therapeutic materials (Kohn, 1999). More importantly, the ability of HSCs to induce immune tolerance to transgenes and their associated proteins makes gene transfer especially suitable to be coupled with BMT (Bordignon and Roncarolo, 2002; Sykes et al., 1993).

Owing to the low frequency of integration, attempts to use adeno-associated viruses (AAVs) to introduce genes into HSCs have largely failed (Miller et al., 1995; Ponnazhagan et al., 1998). Thus, the efforts have been mainly focused on oncoretrovirus- and lentivirus-mediated gene transfer into HSCs (Bauer et al., 1994; Hanenberg et al., 1997; Leurs et al., 2003; Miller, 1992a; Miller, 1992b; Miller, 2003; Miller et al., 1993; Walsh et al., 1993; Williams, 1990; Williams and Smith, 2000; Wilson et al., 1997). These viruses share some features (Mulligan, 1993; Varmus, 1988; Varmus, 1982) which are crucial for adaptation to HSC gene transfer, namely: recognition of target cells, achieved by interaction between the envelope protein of the virus and the

specific molecules on cell surface; reverse transcription, which must occur to synthesize the cognate DNA, which is mainly controlled by cytoplasmic concentration of deoxynucleotide triphosphates (dNTPs); the resulting DNA entering the nucleus and integrating into the host genome, which is catalyzed by integrase packaged by the virus.

The Moloney Murine Leukemia Viruses (MMuLVs), which can cause leukemia in mice, were the first retroviruses to be used for gene therapy (Miller, 1992b). As mentioned, the envelope protein determines the specificity of the virus. Currently the envelope protein of ecotropic retroviruses (Eco) is popularly used to package MMuLVs to infect mouse HSCs, because Eco can only recognize a murine basic amino acid transport protein (mCAT) and has no capability to infect human cells, providing the safety of use in laboratories (Albritton et al., 1993; Albritton et al., 1989; Davey et al., 1997; Fass et al., 1997; Kim et al., 1991). For transduction of human HSCs, the envelope protein from amphotropic retroviruses, which recognizes a phosphate transporter protein, Pit-2, is dominantly used (Miller et al., 1994; Mulligan, 1993). The envelope protein from Vesicular Stomatitis Virus type G (VSV-G) binds to a cell membrane phospholipid, so viruses packaged by VSV-G can infect virtually any type of cells (Burns et al., 1993; Schlegel et al., 1983; Yee et al., 1994). It should be noted that one unique property of VSV-G is that the packaged viruses can be concentrated to have very high titers (Chen et al., 1996; Ory et al., 1996; Yang et al., 1995).

Several versions of recombinant oncoretrovirus vectors have been engineered. LTR units from different viruses have the distinct capacity to induce protein expression in different cell types (Miller, 1992b). For instance, LTRs from MMuLVs are particularly active in lymphoid cells, while LTRs derived from Mouse Stem Cell Viruses (MSCVs) generally promote high protein expression in both myeloid and lymphoid cells (Beckengeser et al., 1991; Onodera et al., 1998; Pawliuk et al., 1996; Pawliuk et al., 1997). The LTRs can also be silenced over time in HSCs (Challita et al., 1995a); appropriate engineering can partially circumvent this problem (Challita et al., 1995b; Robbins et al., 1997). Interestingly, the LTR from MSCV exhibits exceedingly good resistance to the gene silencing (Cheng et al., 1998).

Unlike oncoretrovirus, lentivirus can integrate transgenes into non-dividing cells (Lewis et al., 1992; Poeschla, 2003). This property offers certain advantages for its application in HSC gene transfer, considering that HSCs are usually quiescent and have prolonged cell cycles. Lentivirus employs its own viral proteins to promote the transport of preintegration complex into the nucleus using host cell nuclear transport machinery (Bukrinsky et al., 1993; Gallay et al., 1995). The most commonly known lentiviruses are the Human Immunodeficiency Virus 1 (HIV1) and 2 (HIV2). VSV-G is the most common envelope proteins used to package lentivirus, although use of Eco and amphotropic envelope was also reported (Naldini et al., 1996; Sutton et al., 1998). A successful example of lentivirus-mediated gene transfer into HSCs was the gene therapy for Sickle Cell Disease in mouse models, in which onco-

retroviral vectors failed to transfer the required gene expression cassette for expressing human β-globin (May et al., 2000; Pawliuk et al., 2001).

Toward the use of retrovirus-mediated gene transfer into HSCs, various protocols have been devised for the application in mouse models. The most refined protocol includes treatment of donor mice with 5-fluorouracil (a cell cycle specific drug that specifically targets to cycling cells like hematopoietic progenitor cells) for 5 days to deplete the progenitor cells and enrich HSCs (Figure 1-7) (Hodgson and Bradley, 1979; Suzuki et al., 2002). Three cytokines (IL-3, IL-6 and SCF) are added into culture to further expand HSCs without losing significant stem cell properties (Bodine et al., 1989; Bodine et al., 1991). These stimulated HSCs are then exposed to retroviruses to receive transgenes (Figure 1-7). If necessary, several rounds of infection can be performed to increase the overall transfection efficiency. The infected HSCs are transferred into lethally or sub-lethally irradiated mice. It takes 4-6 weeks for the recipients to reconstitute the entire hematopoietic system.

HSC gene therapy has been employed in animal models to evaluate the feasibility of this approach to treat severe combined immune deficiencies (Belmont et al., 1988; Wilson et al., 1990), inborn errors of metabolism (Correll et al., 1990a), and hemoglobinopathic genes (Bender et al., 1989; Bender et al., 1988; Correll et al., 1990b; Dzierzak et al., 1988). Birkenmeier and co-workers reported the first successful example of HSC gene transfer to treat human Sly Syndrome (β-glucuronidase deficiency) in a mouse model (Wolfe et al., 1992). To date, successful treatment of several human diseases has been

demonstrated in animal models. These diseases include X-SCID, caused by the deficiencies of the Jak3 and common γ-chains (Bunting et al., 1998b; Lo et al., 1999; Mendoza et al., 2001; Otsu et al., 2000; Otsu et al., 2001), Chronic Granulomatous Disease (CGD) caused either by gp91phox or p47phox deficiency (Bjorgvinsdottir et al., 1997; Mardiney et al., 1997), etc.

The safety issue of retrovirus-mediated gene transfer approach has been subjected to intense discussion and debate. In mice, it is well documented that insertion of LTR elements into oncogenic sites have the possibility to cause leukemogenesis (Corcoran et al., 1984; Suzuki et al., 2002). Over thousands of BMT experiments using either oncoretroviruses or lentiviruses in mice, the occurrence of abnormal growth of hematopoietic cells was observed in two cases (Bunting et al., 1998a; Li et al., 2002). It was estimated that the chance to have leukemia causing insertion by retrovirus-mediated gene transfer was less than 1/10,000 (Mucenski et al., 1988). The safety concern was dramatically raised when two out of 9 children who receiving gene therapy for the X-SCID disease developed leukemia (Cavazzana-Calvo et al., 2000; Hacein-Bey-Abina et al., 2003; Kaiser, 2003; Marshall, 2003). In the trial, the French team was able to restore the immune system for 9 out of 11 children with X-SCID disease, showing the first successful example of human gene therapy. The clinical trial used retroviruses to deliver the gene for common γ chain into patients via HSC transplantation to correct the deficient gene. The first observed leukemia case involved in replication of a single $\gamma\delta$ T cell, while the second case involved in over-production of three types of an $\alpha\beta$ T cell. Further analysis revealed that the

retroviral vector was inserted into a site close to *LMO2* gene in both cases, which was a known proliferative gene (Hacein-Bey-Abina et al., 2003). The retrovirus-activated *LMO2* gene could cooperate with the transduced γc gene to promote the over-growth of the inserted clone. Many lessons were learned from this trial. In future studies, safer strategies have to be devised, including incorporation of insulator elements in the vector to prevent induction of up- or down-stream associated genes, use of tissue-specific or more controllable promoters, inclusion of suicide genes, etc. This trial emphasizes the importance of continuous development of animal models to evaluate every possible aspect of safety issues involved in HSC gene therapy.

5. The Role of IL-2 on T Cell Regulatory Function and Memory

IL-2 is one of the major cytokines to regulate T cell function. It is an important growth factor to mediate T cell proliferation upon antigenic stimulation; it also can promote T cell survival by elevating expression level of anti-apoptotic molecules such as Bcl-2 (Akbar et al., 1996; Gillis and Smith, 1977; Van Parijs et al., 1999). In view of its positive role, IL-2 has been used in immunotherapy to enhance immune response against cancer (Waldmann, 2003). In fact, IL-2 was the first cytokine to receive approval from the US Food and Drug Administration (FDA) for the treatment of metastatic renal cancer and malignant melanoma (Atkins et al., 1999; Rosenberg et al., 1994). The role of IL-2 in immune system was expanded when investigators discovered that IL-2 knockout mice, although mounting a reduced immune response towards viral infection, developed

autoimmune disease (Sadlack et al., 1993). Similarly, targeted disruption of the high affinity IL-2 receptor gene led to the accumulation of activated T cells and autoimmunity (Suzuki et al., 1995; Willerford et al., 1995). These observations suggest that IL-2 has important roles in maintaining peripheral tolerance. The exact mechanism on how IL-2 controls the expansion of T cells is not completely understood, and one possibility is that IL-2 can render activation induced cell death (AICD), which is likely regulated through Fas-mediated apoptosis (Lenardo, 1996). This may limit the therapeutic impact that IL-2 can generate; IL-2 can instruct the tumor responsive T cells to recognize tumors as self-antigens and promote AICD-induced apoptosis (Waldmann, 2003). More experiments are required to further confirm this speculation.

The fact that IL-2 receptor α chain (IL-2Rα, CD25) is consistently upregulated in $CD4^+CD25^+$ T cells suggested that IL-2 have roles in regulatory T cell (Treg) biology (Nelson, 2004). Malek and co-workers expressed IL-2Rβ transgene in IL-2Rβ knockout mice with selective expression only in the thymus and found that this transgene could rescue $CD4^+CD25^+$ T cell development, suggesting that IL-2 is required for Treg development in thymus (Malek et al., 2000; Malek et al., 2002). To investigated the role of IL-2 signaling in peripheral, the same group also transferred $CD4^+CD25^+$ T cells from wild-type mice into IL-2 deficient mice and found that transferred Tregs failed to expand, indicating that IL-2 is also required for Treg expansion and maintenance in periphery (Malek et al., 2002). Several lines of evidence showed that Tregs treated with IL-2 reduced their suppressive activity, and Tregs lacking IL-2Rβ gene could still exert

negative regulation to prevent autoimmunity, implying that IL-2 is not required for Tregs to maintain regulatory function (Bach, 2003; Gavin et al., 2002; Malek et al., 2002). Nevertheless, the involvement of IL-2 in Tregs has been firmly demonstrated, although the detailed molecular mechanisms are still controversial.

It is increasingly clear that cytokines are key molecular mediators in regulation of generation and maintenance of T cell memory (Schluns and Lefrancois, 2003). For CD8 T cells, it has become clear that the members of the common cytokine receptor γ chain (γc) family, including interleukin 2 (IL-2), IL-7 and IL-15, contribute significantly to memory T cell production and differentiation (Schluns and Lefrancois, 2003). Notably IL-2 was found to have a dual role in development of CD8 memory: IL-2 increased the population of the CD8 memory if present at the initial activation of T cells, but decreased the size if present during memory maintenance (Dai et al., 2000). In sharp contrast, Ahmed and co-workers evaluated the effects of IL-2 treatment during the expansion, contraction and memory phases of the CD8 T cell responses (Blattman et al., 2003) and revealed that IL-2 treatment at the expansion phase has a negative effect on the survival of effector CD8 T cells; treatment of IL-2 at the death phase can increase the proliferation and survival of antigen-specific effector CD8 T cells. When IL-2 was used to treat the resting memory CD8 T cells, rapid expansion of memory cells was observed, indicative of a positive effect of IL-2 on memory maintenance. Further studies are required to resolve these controversial observations on the role of IL-2 in T cell memory. .

6. Research Objectives and Thesis Outline

Towards engineering immunity, this thesis focuses on T cell immunotherapy. We propose a concept of instructive immunotherapy for cancer, as compared to active and passive immunotherapy. In the context of cancer therapy, active immunotherapy involves activating the effectors in the host immune system to inhibit cancer cell growth and reject tumors (e.g., cancer vaccination), while passive immunotherapy is a term for directly providing the host with the effectors to react against cancer (e.g., adoptive transfer of in vitro expanded antitumor T cells). Our concept of instructive immunotherapy is to use a strategy to guide the host in developing in vivo effector cells capable of targeting cancer. This strategy arises from combination of gene therapy, stem cell therapy and immunotherapy to program HSCs to develop into lymphocytes with desired antitumor specificity. The choice of HSCs endows this method a unique feature. Taking advantage of the longevity and self-renewal of HSCs, life-long supplies of tumor-specific lymphocytes can be generated in vivo, which exceed the current methods of repetitive immunization and adoptive transfer.

Following the introduction chapter 1, I will devote the second chapter to test the feasibility of this approach. Retrovirus-mediated gene transfer of cDNA into RAG-deficient hematopoietic precursor cells allows the long-term production of functional antigen-specific T cells. Chapter 3 describes the results of imparting antitumor specificity to T cell repertoire via bone marrow transplantation of programmed wild-type HSCs. Co-expression of α and β chains of CD8-specific T

cells permitted efficient production of desired antitumor CTLs with an occupation of approximately 20% of total CD8 T cell repertoire. Significant tumor rejection was observed in mice, demonstrating the feasible approach of instructive cancer immunotherapy. In recognition of the important roles of helper T cells in antitumor immunity, chapter 4 elaborates a two-arm model to augment tumor-specific immune responses. In the experiment, the two arms, both antitumor CD4- and CD8 T cells, were generated by HSC gene transfer method. The resultant immune system in mice can not only suppress tumor growth, but also eradicate large, solid and vascularized tumors.

Successful immunotherapy relies on understanding the molecular mechanisms that control immune responses. For instance, although IL-2 has been approved by FDA to treat renal cancer and melanoma, many results from mice show that the physiological role of IL-2 is complex and unpredictable, hindering the design of better strategies which would maximize the therapeutic impact of IL-2. I address the role of IL-2 in negative regulatory function and T cell memory in last two chapters, both of which are important for achieve the overall success of immunotherapy. Chapter 5 describes the role of IL-2 in maintaining regulatory T cell homeostasis and self-tolerance, and correlates this role with the signaling molecule STAT5. The final chapter (Chapter 6) details the role of IL-2 in generation and maintenance of CD4 T cell memory.

Table 1-1: History of immunotherapy (adapted from *Nat. Med*, 9, 269 (2003))

Time	Milestone Accomplishments
1796	Jenner introduces vaccinia (cowpox) immunization to prevent subsequent smallpox infection
1879-1886	Louis Pasteur introduces first laboratory-weakened infectious agent (chicken cholera bacterium) and strongly thereafter develops weakened rabies for active immunization
1888	Emile Roux and Alexandre Yersin isolate toxin from diphtheria
1890	Emil von Behring and Shibasabo Kitasato in Koch's laboratory find that injecting diphtheria toxin into animals produces a serum containing an antitoxin that provides passive anti-diphtheria immunity to people
1900	Paul Ehrlich suggests that molecules that react with tumors could play a role in cancer therapy, presaging antibody-mediated passive immunity
1954-1955	Jonas Salk and Albert Sabin introduce killed and live attenuated polio vaccines that soon lead to the elimination of poliomyelitis
1965	IgG anti-D (anti-RH) is administrated to prevent of RH immunization and thus prevent erythroblastosis fetails; this is a translation of the basic insight that passive administration of a specific IgG antibody inhibits the active production of that antibody
1975	George Kohler and Cesar Milstein develop hybridoma technology for monoclonal antibody generation
1977	Smallpox is declared eradicated through vaccination
1982	The first report of successful use of a monoclonal antibody to treat a human neoplasm is reported
1986	The first monoclonal antibody, muromonab-CD3 (Orthoclone OKT3), is approved by FDA
1986	The first humanized antibody is produced by replacing the complementarity regions in a human body with those of mouse
1986-2000	IL-2, IFN-α, IFN-β and IFN-γ are approved for use in the treatment of neoplasia, hepatitis and multiple sclerosis
1988-1991	The methodology for isolating tumor antigens recognized by CTLs is introduced; the first human antigen from melanoma patients identified by CTLs is isolated
1997	The first humanized monoclonal antibody (daclizumab, Zenapax) is approved by the FDA
1997	The first monoclonal antibody (rituximab, Rituxan) for the treatment of malignancy is approved
1998	An antibody to TNF-a (infliximab, Rituxan), and p75 TNF receptor linked to the Fc or IgG1 (etanercept, Enbrel) are approved for use in the treatment of rheumatoid arthritis and Crohn disease
2000	The first toxin-linked monoclonal antibody (gemtuzumab ozogamicin Mylotarg) is approved by the FDA
2002	The first radionuclide-linked monoclonal antibody (ibritumomab tiuxetan, Zevalin) is approved by the FDA

Table 1-2: Mechanisms that account for failure of efficient immune responses against tumors (adapted from *Nat. Rev. Cancer*, 3, 667 (2003))

	Possible Mechanisms
The generation phase of anti-tumor immune responses	Lack of T cell help Non-activated APCs Active suppression by regulatory T cells
The tumor-reactive $CD8^+$ lymphocytes	Insufficient number of tumor-antigen-specific precursor CTLs Insufficient avidity of T cells for tumor antigens Downregulation of T cell receptor signal transduction Downregulation of T cell receptor for tumor antigens Inadequate T cell function (cytokine, lysis)
The effector phase of the antitumor immune response	Apoptosis of T cells when encountering tumor T cell failure to extravasate from vasculature to tumor stroma Tumor produces local immuno-suppressive factors
The tumor cells	Loss of tumor-antigen expression Loss of HLA expression Resistance to CTL lysis Loss of apoptotic or other self-destructive functions

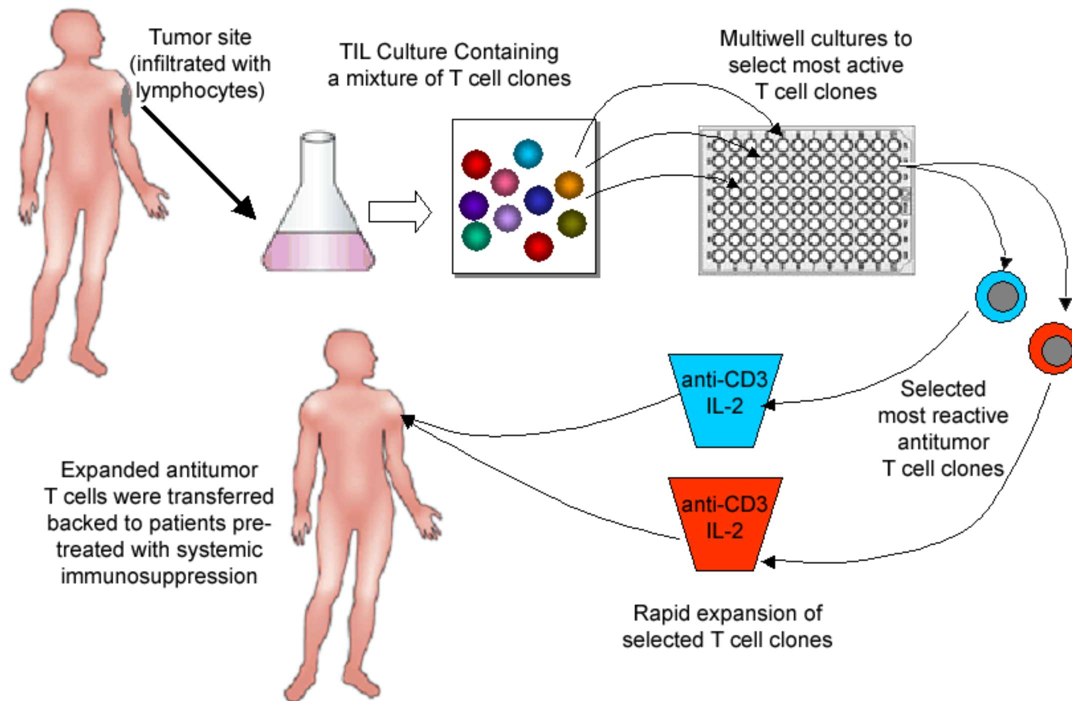

Figure 1-1: A schematic presentation of adoptive T cell transfer therapy for patients with cancer.

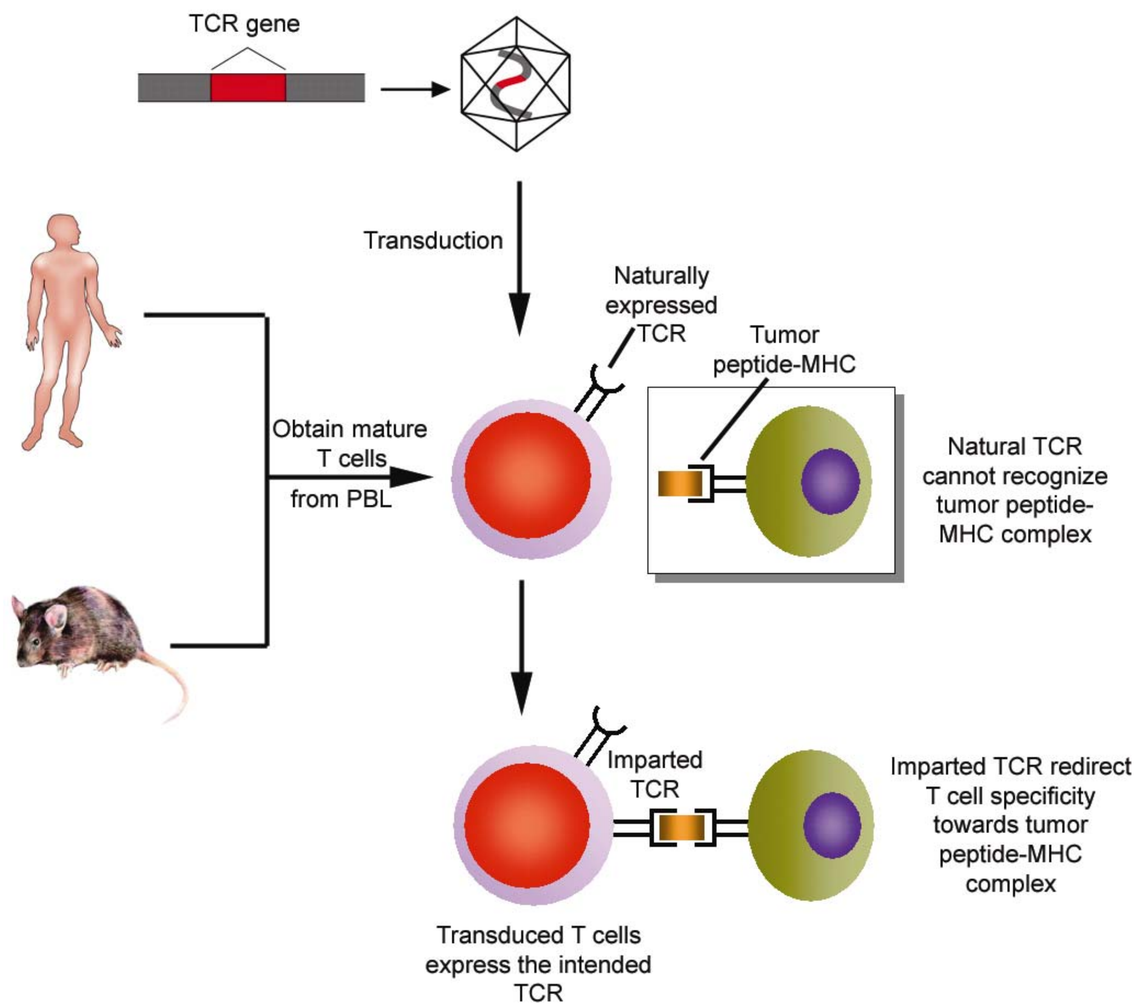

Figure 1-2: Engineering redirected antitumor specificity into mature T cells from PBL via retrovirus-mediated gene transfer of TCR cDNA genes.

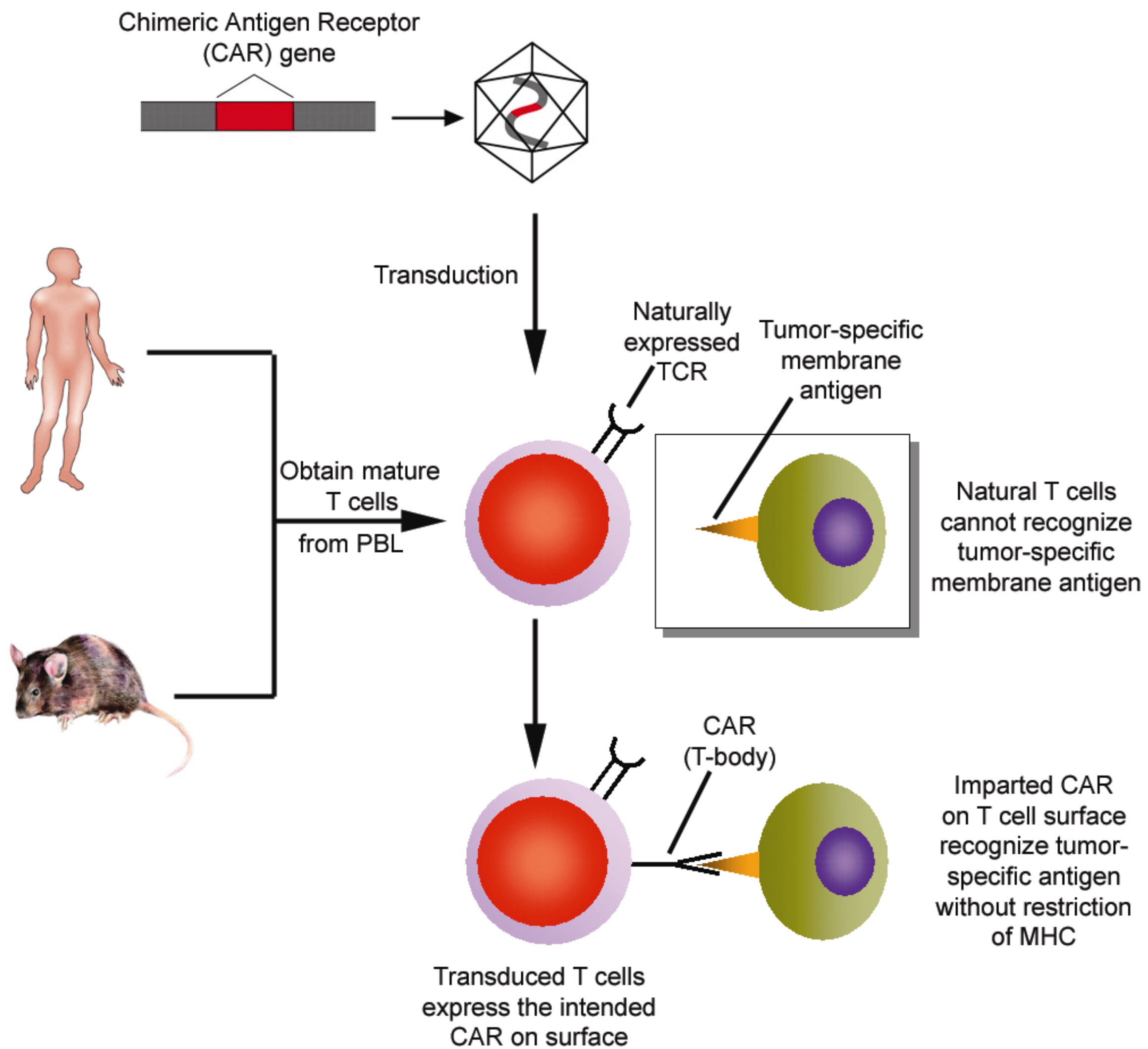

Figure 1-3: Engineering mature T cells from PBL with antitumor specificity by retrovirus-mediated gene transfer of chimeric antigen receptor (recombinant antigen receptor or T-body). The resulting T cells have antitumor reactivity without restriction of MHC.

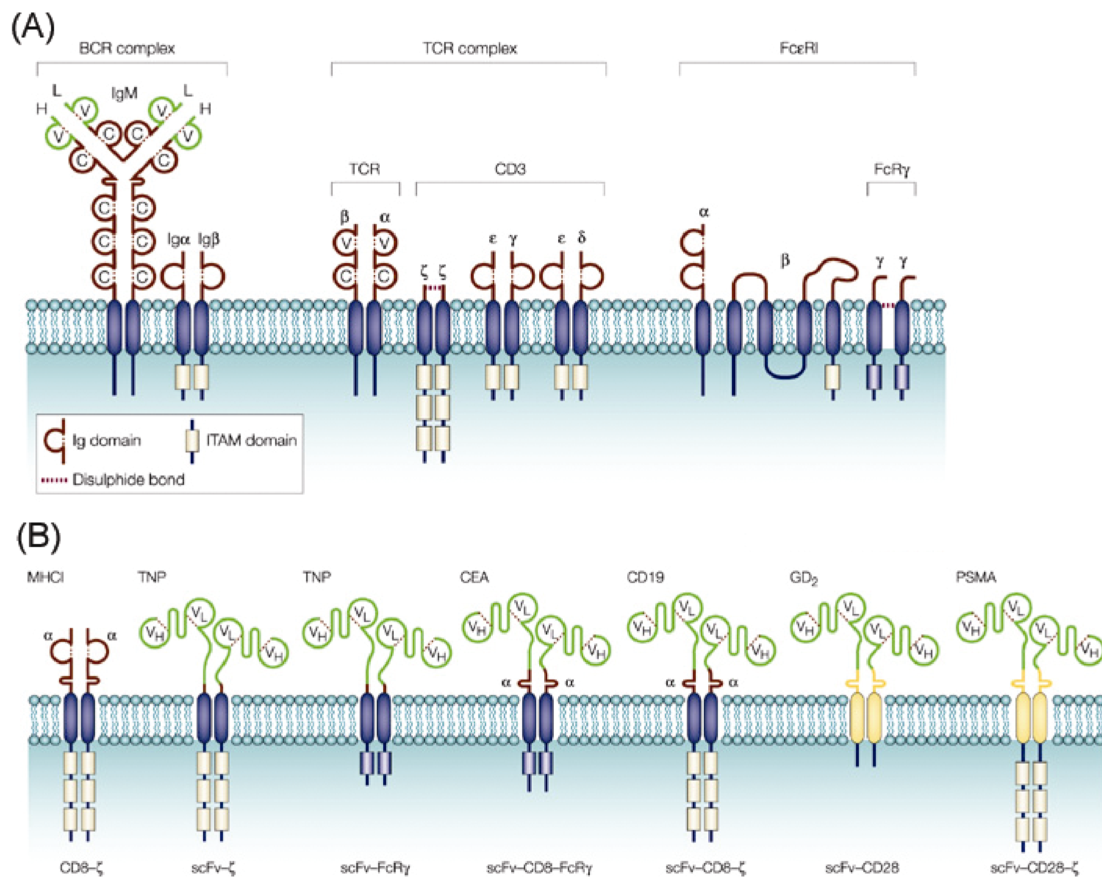

Figure 1-4: (A) Cartoon structure of natural antigen receptors: B cell receptor (BCR), T cell receptor (TCR) and Rc R1 receptor. (B) Cartoon structure of representative chimera antigen receptors. (Adapted from *Nat. Rev. Cancer*, 3, 35 (2003))

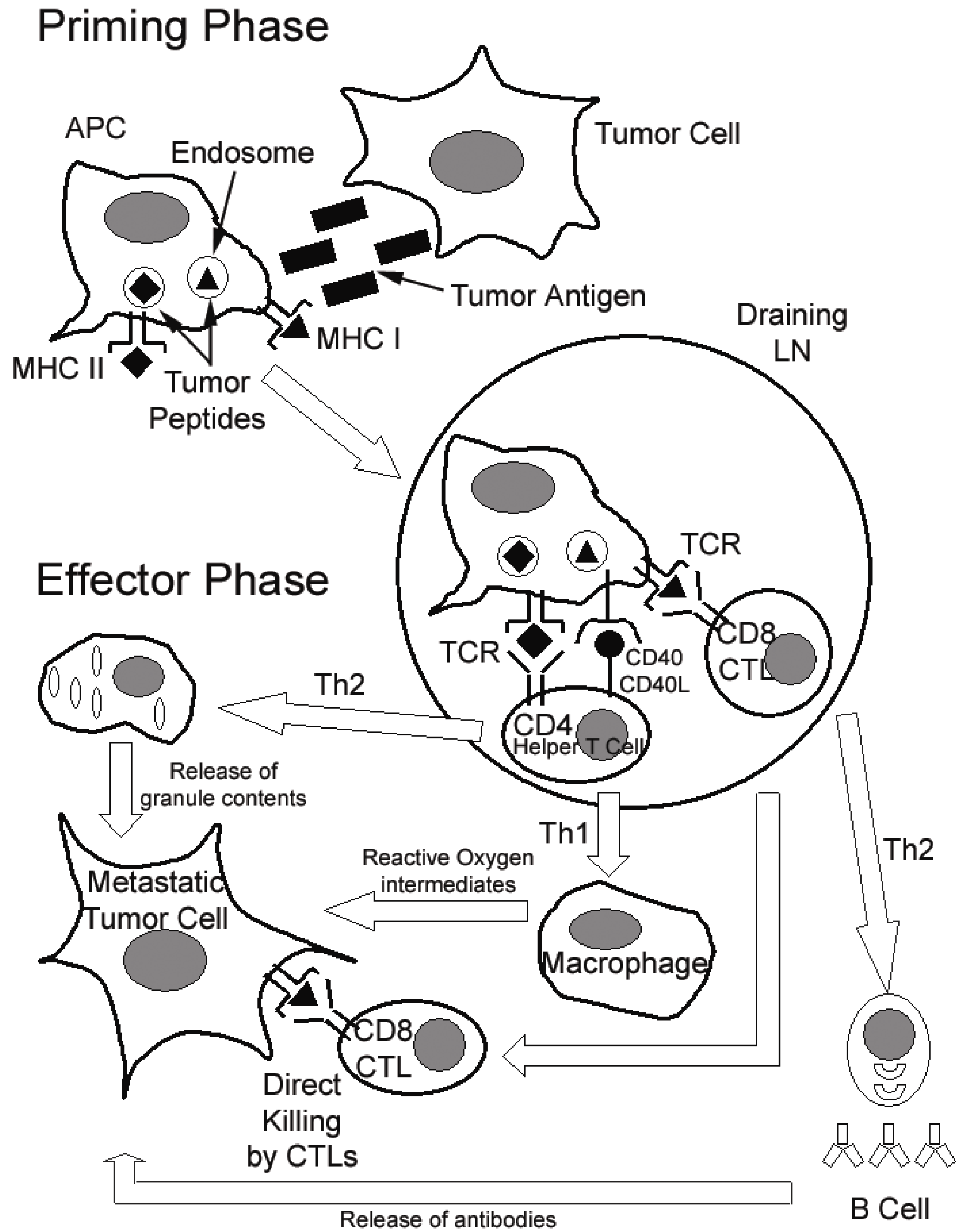

Figure 1-5: The schematic presentation of the model for CD4 T cells to orchestrate multiple effector arms of antitumor immunity (modified from *Curr. Opin. Immunol.*, 10, 588 (1998)).

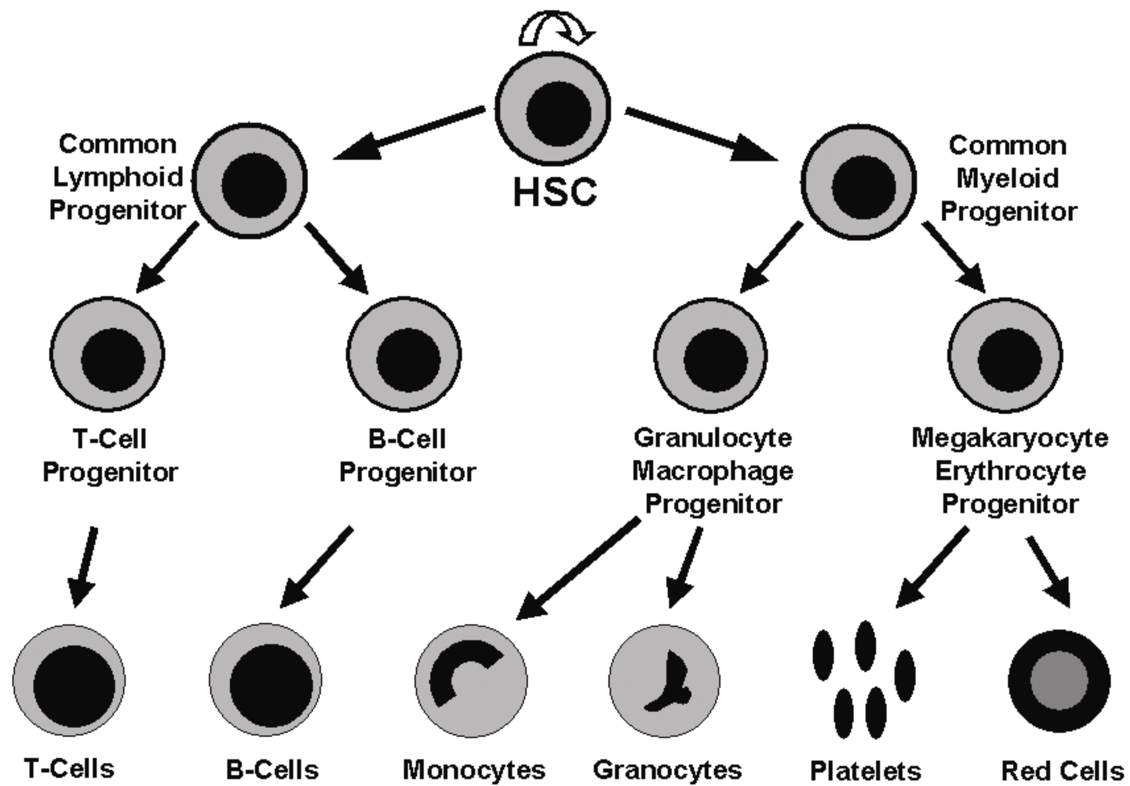

Figure 1-6: The schematic presentation of a model for multipotent HSCs to differentiate into different cells in hematopoietic system.

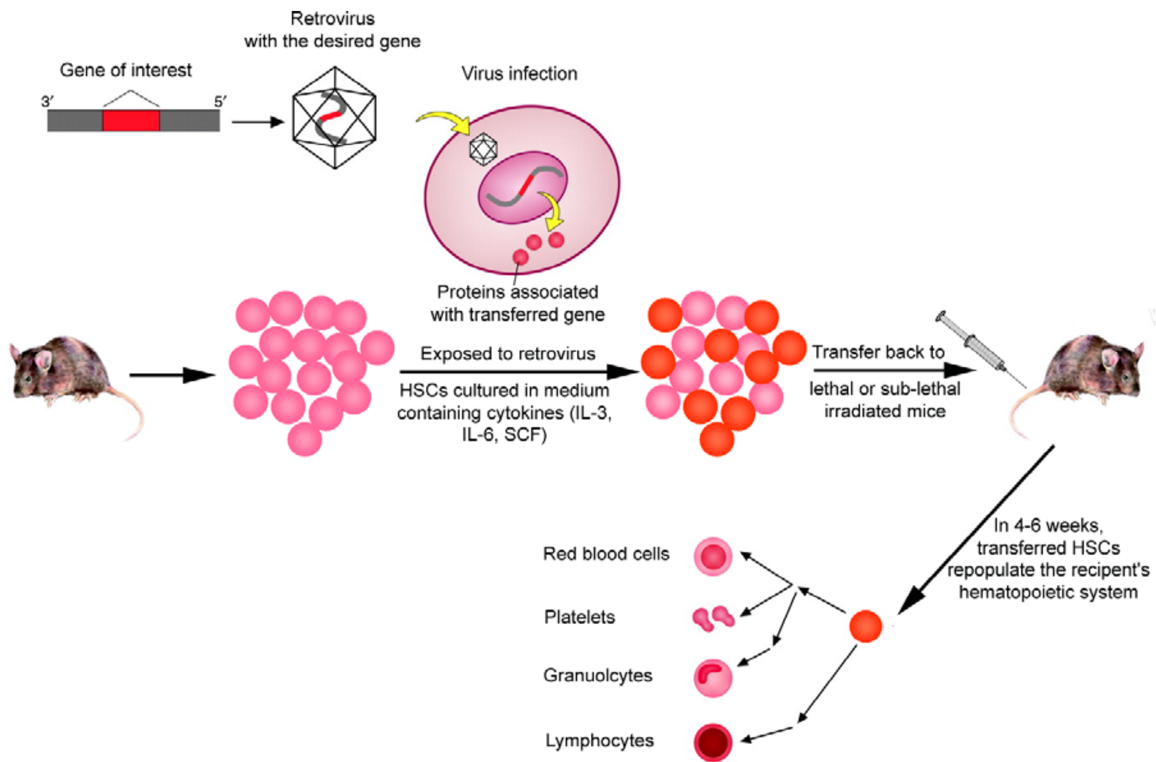

Figure 1-7: The schematic representation of bone marrow transplantation of genetic modified HSCs into lethal or sub-lethal irradiated mice to regenerate the hematopoietic system.

7. References:

Abken, H., Hombach, A., and Heuser, C. (2003). Immune response manipulation: Recombinant immunoreceptors endow T-cells with predefined specificity. Curr Pharm Design *9*, 1992-2001.

Ahlers, J. D., Takeshita, T., Pendleton, C. D., and Berzofsky, J. A. (1997). Enhanced immunogenicity of HIV-1 vaccine construct by modification of the native peptide sequence. Proc Natl Acad Sci U S A *94*, 10856-10861.

Akbar, A. N., Borthwick, N. J., Wickremasinghe, R. G., Panayiotidis, P., Pilling, D., Bofill, M., Krajewski, S., Reed, J. C., and Salmon, M. (1996). Interleukin-2 receptor common gamma-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: Selective induction of anti-apoptotic (bcl- 2, bcl-x(L)) but not pro-apoptotic (bax, bcl-x(S)) gene expression. Eur J Immunol *26*, 294-299.

Albritton, L. M., Kim, J. W., Tseng, L., and Cunningham, J. M. (1993). Envelope-binding domain in the cationic amino acid transporter determines the host range of ecotropic murine retroviruses. J Virol *67*, 2091-2096.

Albritton, L. M., Tseng, L., Scadden, D., and Cunningham, J. M. (1989). A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane spanning protein and confers susceptibility to virus infection. Cell *57*, 659-666.

Altenschmidt, U., Klundt, E., and Groner, B. (1997). Adoptive transfer of in vitro-targeted, activated T lymphocytes results in total tumor regression. J Immunol *159*, 5509-5515.

Amos, T. A. S., and Gordon, M. Y. (1995). Sources of human hematopoietic stem cells for transplantation. Cell Transplant *4*, 547-569.

Anderson, W. F. (1984). Prospects for human gene therapy. Science *226*, 401-409.

Anderson, W. F. (1992). Human gene therapy. Science *256*, 808-813.

Atkins, M. B., Lotze, M. T., Dutcher, J. P., Fisher, R. I., Weiss, G., Margolin, K., Abrams, J., Sznol, M., Parkinson, D., Hawkins, M., *et al.* (1999). High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985 and 1993. J Clin Oncol *17*, 2105-2116.

Bach, J. F. (2003). Regulatory T cells under scrutiny. Nat Rev Immunol *3*, 509-509.

Baselga, J., Norton, L., Albanell, J., Kim, Y. M., and Mendelsohn, J. (1998). Recombinant humanized anti-HER2 antibody (Herceptin (TM)) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res *58*, 2825-2831.

Baselga, J., Norton, L., Albanell, J., Kim, Y. M., and Mendelsohn, J. (1999). Recombinant humanized anti-HER2 antibody (Herceptin (TM)) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts (vol 58, pg 2825, 1998). Cancer Res *59*, 2020-2020.

Bauer, T. R., Miller, A. D., and Hickstein, D. D. (1994). Retroviral vectors and packaging cells lines for use in gene therapy of leukocyte adherence deficiency. Blood *84*, A256-A256.

Beckengeser, G., Stocking, C., Just, U., Albritton, L., Dexter, M., Spooncer, E., and Ostertag, W. (1991). Retroviral vectors related to the myeloproliferative sarcoma virus allow efficient expression in hematopoietic stem and precursor cell lines, but retroviral infection is reduced in more primitive cells. Hum Gene Ther *2*, 61-70.

Beecham, E. J., Ma, Q. Z., Ripley, R., and Junghans, R. P. (2000a). Coupling CD28 co-stimulation to immunoglobulin T-cell receptor molecules: The dynamics of T-cell proliferation and death. J Immunother *23*, 631-642.

Beecham, E. J., Ortiz-Pujols, S., and Junghans, R. P. (2000b). Dynamics of tumor cell killing by human T lymphocytes armed with an anti-carcinoembryonic antigen chimeric immunoglobulin T-cell receptor. J Immunother *23*, 332-343.

Belmont, J. W., Macgregor, G. R., Wagersmith, K., Fletcher, F. A., Moore, K. A., Hawkins, D., Villalon, D., Chang, S. M. W., and Caskey, C. T. (1988). Expression of human adenosine deaminase in murine hematopoietic cells. Mol Cell Biol *8*, 5116-5125.

Bender, M. A., Gelinas, R. E., and Miller, A. D. (1989). A majority of cice show long term expression of a human beta-globin gene after retrovirus transfer into hematopoietic stem cells. Mol Cell Biol *9*, 1426-1434.

Bender, M. A., Miller, A. D., and Gelinas, R. E. (1988). Expression of the human beta-globin gene after retroviral transfer into murine erythroleukemia cells and human Bfu-E cells. Mol Cell Biol *8*, 1725-1735.

Bennett, S. R. M., Carbone, F. R., Karamalis, F., Flavell, R. A., Miller, J., and Heath, W. R. (1998). Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature *393*, 478-480.

Bennett, S. R. M., Carbone, F. R., Karamalis, F., Miller, J., and Heath, W. R. (1997). Induction of a CD8 cytotoxic T lymphocyte response by cross-priming requires cognate CD4 T cell help. J Exp Med *186*, 65-70.

Berzofsky, J. A. (1993). Ann NY Acad Sci *690*, 256-264.

Bjorgvinsdottir, H., Ding, C. J., Pech, N., Gifford, M. A., Li, L. L., and Dinauer, M. C. (1997). Retroviral-mediated gene transfer of gp91(phox) into bone marrow cells rescues defect in host defense against Aspergillus fumigatus in murine X-linked chronic granulomatous disease. Blood *89*, 41-48.

Blattman, J. N., Grayson, J. M., Wherry, E. J., Kaech, S. M., Smith, K. A., and Ahmed, R. (2003). Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. Nature Medicine *9*, 540-547.

Bock, T. A., Dunbar, C. E., Orlic, D., and Bodine, D. M. (1993). Transgenic scid mice containing the human IL-3, GM-CSF, and SCF genes as a model for human hematopoiesis. Blood *82*, A457-A457.

Bock, T. A., Orlic, D., Dunbar, C. E., Broxmeyer, H. E., and Bodine, D. M. (1995). Improved engraftment of human hematopoietic cells in severe combined immunodeficient (SCID) mice carrying human cytokine transgenes. J Exp Med *182*, 2037-2043.

Bodine, D. M., Dunbar, C. E., Girard, L. J., Seidel, N. E., Cline, A. P., Donahue, R. E., and Orlic, D. (1998). Improved amphotropic retrovirus-mediated gene transfer into hematopoietic stem cells. In Cooleys Anemia, pp. 139-150.

Bodine, D. M., Karlsson, S., and Nienhuis, A. W. (1989). Combination of interleukin-3 and interleukin-6 preserves stem cell function in culture and enhances retrovirus-dediated gene-transfer into hematopoietic stem cells. Proc Natl Acad Sci U S A *86*, 8897-8901.

Bodine, D. M., McDonagh, K. T., Seidel, N. E., and Nienhuis, A. W. (1991). Survival and retrovirus infection of murine hematopoietic stem cells in vitro: effects of 5-FU and method of infection. Exp Hematol *19*, 206-212.

Bodine, D. M., Seidel, N. E., Cline, A. P., Gale, M. S., and Orlic, D. (1995). The repopulating ability of bone marrow from mice treated with the combination of granulocyte colony stimulating factor (G- CSF) and stem cell factor (SCF) is increased approximately 15 fold. Blood *86*, 893-893.

Bodine, D. M., Seidel, N. E., Gale, M. S., Nienhuis, A. W., and Orlic, D. (1994). Efficient retrovirus transduction of mouse pluripotent hematopoietic stem cells mobilized into the peripheral blood by treatment with granulocyte-colony-stimulating factor and stem cell factor. Blood *84*, 1482-1491.

Bodine, D. M., Seidel, N. E., and Orlic, D. (1996). Bone marrow collected 14 days after in vivo administration of granulocyte colony-stimulating factor and stem cell factor to mice has 10-fold more repopulating ability than untreated bone marrow. Blood *88*, 89-97.

Boggs, D. R., Boggs, S. S., Saxe, D. F., Gress, L. A., and Canfield, D. R. (1982). Hematopoietic stem cells with high proliferative potential: Assay of their concentration in marrow by the frequency and duration of cure of W/Wv mice. J Clin Invest *70*, 242-253.

Bordignon, C., and Roncarolo, M. G. (2002). Therapeutic applications for hematopoietic stem cell gene transfer. Nat Immunol *3*, 318-321.

Brasel, K., McKenna, H. J., Charrier, K., Morrissey, P., Williams, D. E., and Lyman, S. D. (1995a). Mobilization of peripheral blood progenitor cells with Flt3 ligand. Blood *86*, 1838-1838.

Brasel, K., McKenna, H. J., Charrier, K., Morrissey, P., Williams, D. E., and Lyman, S. D. (1995b). Synergistic effects in vivo of FLT3 ligand with GM-CSF or G-CSF in mobilization of colony forming cells in mice. Blood *86*, 1986-1986.

Brasel, K., McKenna, H. J., Charrier, K., Morrissey, P. J., Williams, D. E., and Lyman, S. D. (1997). Flt3 ligand synergizes with granulocyte-macrophage colony-stimulating factor or granulocyte colony-stimulating factor to mobilize hematopoietic progenitor cells into the peripheral blood of mice. Blood *90*, 3781-3788.

Brasel, K., McKenna, H. J., Morrissey, P. J., Charrier, K., Morris, A. E., Lee, C. C., Williams, D. E., and Lyman, S. D. (1996). Hematologic effects of flt3 ligand in vivo in mice. Blood *88*, 2004-2012.

Brentjens, R. J., Latouche, J. B., Riviere, I., and Sadelain, M. (2002). In vivo anti-tumor activity of genetically modified T cells is dependent on the method of ex vivo T cell expansion. Blood *100*, 577A-577A.

Brown, J. M., Weissman, I. L., and Shizuru, J. A. (2001). Immunity to infections following hematopoietic cell transplantation. Curr Opin Immunol *13*, 451-457.

Broxmeyer, H. E. (1995). Questions to be answered regarding umbilical-cord blood hematopoietic stem and progenitor cells and their sse in transplantation. Transfusion *35*, 694-702.

Bukrinsky, M. I., Haggerty, S., Dempsey, M. P., Sharova, N., Adzhubei, A., Spitz, L., Lewis, P., Goldfarb, D., Emerman, M., and Stevenson, M. (1993). A nuclear localization signal within HIV-1 matrix protein that governs infection of nondividing cells. Nature *365*, 666-669.

Bunting, K. D., Galipeau, J., Topham, D., Benaim, E., and Sorrentino, B. P. (1998a). Transduction of murine bone marrow cells with an MDR1 vector enables ex vivo stem cell expansion, but these expanded grafts cause a myeloproliferative syndrome in transplanted mice. Blood *92*, 2269-2279.

Bunting, K. D., Sangster, M. Y., Ihle, J. N., and Sorrentino, B. P. (1998b). Restoration of lymphocyte function in Janus kinase 3-deficient mice by retroviral-mediated gene transfer. Nature Medicine *4*, 58-64.

Burns, J. C., Friedmann, T., Driever, W., Burrascano, M., and Yee, J. K. (1993). Vesicular stomatitis-virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proc Natl Acad Sci U S A *90*, 8033-8037.

Cavallo, F., Giovarelli, M., Gulino, A., Vacca, A., Stoppacciaro, A., Modesti, A., and Forni, G. (1992). Role of neutrophils and CD4 lymphocytes in the primary and memory response to nonimmunogenic murine mammary adenocarcinoma made immunogenic by IL-2 gene. J Immunol *149*, 3627-3635.

Cavazzana-Calvo, M., Hacein-Bey, S., Basile, C. D., Gross, F., Yvon, E., Nusbaum, P., Selz, F., Hue, C., Certain, S., Casanova, J. L.*, et al.* (2000). Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science *288*, 669-672.

Cella, M., Scheidegger, D., PalmerLehmann, K., Lane, P., Lanzavecchia, A., and Alber, G. (1996). Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T help via APC activation. J Exp Med *184*, 747-752.

Celluzzi, C. M., and Falo, L. D. (1997). Immunization with dendritic cells fused with tumor cells elicits potent tumor specific immunity. J Invest Dermatol *108*, 157-157.

Celluzzi, C. M., and Falo, L. D. (1998). Cutting edge: Physical interaction between dendritic cells and tumor cells results in an immunogen that induces protective and therapeutic tumor rejection. J Immunol *160*, 3081-3085.

Challita, P. M., Skelton, D., Elkhoueiry, A., Yu, X. J., Weinberg, K., and Kohn, D. B. (1995a). Multiple modifications in cis-elements of the long terminal repeat of retroviral vectors lead to increased expression and decreased DNA methylation in embryonic carcinoma cells. J Virol *69*, 748-755.

Challita, P. M., Skelton, D., Elkhoueiry, A., Yu, X. J., Weinberg, K., and Kohn, D. B. (1995b). Multiple Modifications in Cis-Elements of the Long Terminal Repeat of Retroviral Vectors Lead to Increased Expression and Decreased DNA Methylation in Embryonic Carcinoma-Cells. Journal of Virology *69*, 748-755.

Chambers, C. A., Kuhns, M. S., Egen, J. G., and Allison, J. P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol *19*, 565-594.

Chames, P., Willemsen, R. A., Rojas, G., Dieckmann, D., Rem, L., Schuler, G., Bolhuis, R. L., and Hoogenboom, H. R. (2002). TCR-like human antibodies expressed on human CTLs mediate antibody affinity-dependent cytolytic activity. J Immunol *169*, 1110-1118.

Chang, A. E., Redman, B. G., Whitfield, J. R., Nickoloff, B. J., Braun, T. M., Lee, P. P., Geiger, J. D., and Mule, J. J. (2002). A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. Clin Cancer Res *8*, 1021-1032.

Chen, S. T., Iida, A., Guo, L., Friedmann, T., and Yee, J. K. (1996). Generation of packaging cell lines for pseudotyped retroviral vectors of the G protein of vesicular stomatitis virus by using a modified tetracycline inducible system. Proc Natl Acad Sci U S A *93*, 10057-10062.

Cheng, L. Z., Du, C. C., Lavau, C., Chen, S., Tong, J., Chen, B. P., Scollay, R., Hawley, R. G., and Hill, B. (1998). Sustained gene expression in retrovirally transduced, engrafting human hematopoietic stem cells and their lympho- myeloid progeny. Blood *92*, 83-92.

Clay, T. M., Custer, M. C., McKee, M. D., Parkhurst, M., Robbins, P. F., Kerstann, K., Wunderlich, J., Rosenberg, S. A., and Nishimura, M. I. (1999a). Changes in the fine specificity of gp100((209-217))-reactive T cells in patients following vaccination with a peptide modified at an HLA-A2.1 anchor residue. J Immunol *162*, 1749-1755.

Clay, T. M., Custer, M. C., Sachs, J., Hwu, P., Rosenberg, S. A., and Nishimura, M. I. (1999b). Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. J Immunol *163*, 507-513.

Clynes, R. A., Towers, T. L., Presta, L. G., and Ravetch, J. V. (2000). Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nature Medicine *6*, 443-446.

Condon, C., Watkins, S. C., Celluzzi, C. M., Thompson, K., and Falo, L. D. (1996). DNA-based immunization by in vivo transfection of dendritic cells. Nature Medicine *2*, 1122-1128.

Cooper, L. J. N., Kalos, M., Lewinsohn, D. A., Riddell, S. R., and Greenberg, P. D. (2000). Transfer of specificity for human immunodeficiency virus type 1 into primary human T lymphocytes by introduction of T-cell receptor genes. J Virol *74*, 8207-8212.

Corcoran, L. M., Adams, J. M., Dunn, A. R., and Cory, S. (1984). Murine T-lymphomas in which the cellular Myc oncogene has been activated by retroviral insertion. Cell *37*, 113-122.

Correll, P. H., Kew, Y., Perry, L. K., Brady, R. O., Fink, J. K., and Karlsson, S. (1990a). Expression of human glucocerebrosidase in long term reconstituted mice following retroviral-mediated gene transfer into hematopoietic stem cells. Hum Gene Ther *1*, 277-287.

Correll, P. H., Kew, Y., Perry, L. K., Brady, R. O., Fink, J. K., and Karlsson, S. (1990b). Expression of Human Glucocerebrosidase in Long-Term Reconstituted Mice Following Retroviral-Mediated Gene-Transfer into Hematopoietic Stem-Cells. Human Gene Therapy *1*, 277-287.

Dai, Z., Konieczny, B. T., and Lakkis, F. G. (2000). The dual role of IL-2 in the generation and maintenance of CD8+ memory T cells. J Immunol *165*, 3031-3036.

Daly, T., Royal, R. E., Kershaw, M. H., Treisman, J., Wang, G., Li, W. P., Herlyn, D., Eshhar, Z., and Hwu, P. (2000). Recognition of human colon cancer by T cells transduced with a chimeric receptor gene. Cancer Gene Ther *7*, 284-291.

Dao, M. A., Tsark, E., and Nolta, J. A. (1999). Animal xenograft models for evaluation of gene transfer into human hematopoietic stem cells. Curr Opin Mol Ther *1*, 553-557.

Darcy, P. K., Haynes, N. M., Snook, M. B., Trapani, J. A., Cerruti, L., Jane, S. M., and Smyth, M. J. (2000). Redirected perforin-dependent lysis of colon carcinoma by ex vivo genetically engineered CTL. J Immunol *164*, 3705-3712.

Davey, R. A., Hamson, C. A., Healey, J. J., and Cunningham, J. M. (1997). In vitro binding of purified murine ecotropic retrovirus envelope surface protein to its receptor, MCAT-1. J Virol *71*, 8096-8102.

Dick, J. E. (1997). Assaying normal and leukemic human stem cells in NOD/SCID mice. Exp Hematol *25*, 4-4.

Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D., and Mulligan, R. C. (1993). Vaccination with Irradiated tumor cells engineered to secrete murine Granulocyte-Macrophage Colony-Stimulating Factor stimulates potent, specific, and long-lasting antitumor immunity. Proc Natl Acad Sci U S A *90*, 3539-3543.

Dudley, M. E., and Rosenberg, S. A. (2003). Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer *3*, 666-U662.

Dudley, M. E., Wunderlich, J. R., Robbins, P. F., Yang, J. C., Hwu, P., Schwartzentruber, D. J., Topalian, S. L., Sherry, R., Restifo, N. P., Hubicki, A. M., *et al.* (2002). Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science *298*, 850-854.

Dunbar, C. E. (1996). Gene transfer to hematopoietic stem cells: Implications for gene therapy of human disease. Annu Rev Med *47*, 11-20.

Dunbar, C. E. (1998). Gene transfer to hematopoietic cells: Progress and problems. Leuk Lymphoma *30*, 15-16.

Dzierzak, E. A., Papayannopoulou, T., and Mulligan, R. C. (1988). Lineage-specific expression of a human beta-globin gene in murine bone-marrow transplant recipients reconstituted with retrovirus-transduced stem cells. Nature *331*, 35-41.

Eaves, C. J., Cashman, J. D., Sutherland, H. J., Otsuka, T., Humphries, R. K., Hogge, D. E., Lansdorp, P. L., and Eaves, A. C. (1991). Molecular analysis of primitive hematopoietic cell proliferation control mechanisms. Ann NY Acad Sci *628*, 298-306.

Ehrlich, P. (1956). On immunity with special reference to cell life. In The Collected Papers of Paul Ehrlich, Vol II: Immunology and Cancer Research, B. Himmelweir, ed. (London, England, Pergammon).

Eshhar, Z., Waks, T., Gross, G., and Schindler, D. G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma-subunit or zeta-subunit of the Immunoglobulin and T cell receptors. Proc Natl Acad Sci U S A *90*, 720-724.

Fass, D., Davey, R. A., Hamson, C. A., Kim, P. S., Cunningham, J. M., and Berger, J. M. (1997). Structure of a murine leukemia virus receptor-binding glycoprotein at 2.0 angstrom resolution. Science *277*, 1662-1666.

Fong, L., Hou, Y. F., Rivas, A., Benike, C., Yuen, A., Fisher, G. A., Davis, M. M., and Engleman, E. G. (2001). Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci U S A *98*, 8809-8814.

Fujio, K., Misaki, Y., Setoguchi, K., Morita, S., Kawahata, K., Kato, I., Nosaka, T., Yamamoto, K., and Kitamura, T. (2000). Functional reconstitution of class II MHC-restricted T cell immunity mediated by retroviral transfer of the alpha beta TCR complex. J Immunol *165*, 528-532.

Gallay, P., Swingler, S., Song, J. P., Bushman, F., and Trono, D. (1995). HIV nuclear import is governed by the phosphotyrosine-mediated binding of matrix to the core domain of integrase. Cell *83*, 569-576.

Gavin, M. A., Clarke, S. R., Negrou, E., Gallegos, A., and Rudensky, A. (2002). Homeostasis and anergy of CD4(+)CD25(+) suppressor T cells in vivo. Nat Immunol *3*, 33-41.

Germain, R. N., and Stefanova, I. (1999). The dynamics of T cell receptor signaling: Complex orchestration and the key roles of tempo and cooperation. Annu Rev Immunol *17*, 467-522.

Gillis, S., and Smith, K. A. (1977). Long term culture of tumor-specific cytotoxic T cells. Nature *268*, 154-156.

Golumbek, P. T., Lazenby, A. J., Levitsky, H. I., Jaffee, L. M., Karasuyama, H., Baker, M., and Pardoll, D. M. (1991). Treatment of established renal cancer by tumor cells engineered to secrete Interleukin-4. Science *254*, 713-716.

Greenberg, P. D. (1991). Adoptive T cell therapy of tumors: Mechanisms operative in the recognition and elimination of tumor cells. Adv Immunol *49*, 281-355.

Greenberg, P. D., Kern, D. E., and Cheever, M. A. (1985). Therapy of disseminated murine leukemia with cyclophosphamide and immune Lyt-1, 2 T cells: Tumor eradication does not require participation of cytotoxic T cells. J Exp Med *161*, 1122-1134.

Guidotti, L. G., and Chisari, F. V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol *19*, 65-91.

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCcormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., *et al.* (2003). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science *302*, 415-419.

Hanenberg, H., Hashino, K., Konishi, H., Hock, R. A., Kato, I., and Williams, D. A. (1997). Optimization of fibronectin-assisted retroviral gene transfer into human CD34(+) hematopoietic cells. Hum Gene Ther *8*, 2193-2206.

Harrison, D. E., Astle, C. M., and Lerner, C. (1988). Number and continuous proliferative pattern of transplanted primitive immunohematopoietic stem cells. Proc Natl Acad Sci U S A *85*, 822-826.

Harty, J. T., Tvinnereim, A. R., and White, D. W. (2000). CD8(+) T cell effector mechanisms in resistance to infection. Annu Rev Immunol *18*, 275-308.

Haynes, N. M., Snook, M. B., Trapani, J. A., Cerruti, L., Jane, S. M., Smyth, M. J., and Darcy, P. K. (2001). Redirecting mouse CTL against colon carcinoma: Superior signaling efficacy of single-chain variable domain chimeras containing TCR-zeta vs Fc epsilon RI-gamma. J Immunol *166*, 182-187.

Haynes, N. M., Trapani, J. A., Teng, M. W. L., Jackson, J. T., Cerruti, L., Jane, S. M., Kershaw, M. H., Smyth, M. J., and Darcy, P. K. (2002). Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. Blood *100*, 3155-3163.

Heiser, A., Maurice, M. A., Yancey, D. R., Wu, N. Z., Dahm, P., Pruitt, S. K., Boczkowski, D., Nair, S. K., Ballo, M. S., Gilboa, E., and Vieweg, J. (2001). Induction of polyclonal prostate cancer-specific CTL using dendritic cells transfected with amplified tumor RNA. J Immunol *166*, 2953-2960.

Ho, W. Y., Blattman, J. N., Dossett, M. L., Yee, C., and Greenberg, P. D. (2003). Adoptive immunotherapy: Engineering T cell responses as biologic weapons for tumor mass destruction. Cancer Cell *3*, 431-437.

Hock, H., Dorsch, M., Diamantstein, T., and Blankenstein, T. (1991). Interleukin-7 induces CD4+ T cell dependent tumor rejection. J Exp Med *174*, 1291-1298.

Hodgson, G. S., and Bradley, T. R. (1979). Properties of hematopoietic stem cells surviving 5-fluorouracil treatment: Evidence for a Pre-cfu-s cell. Nature *281*, 381-382.

Holler, P. D., Holman, P. O., Shusta, E. V., O'Herrin, S., Wittrup, K. D., and Kranz, D. M. (2000). In vitro evolution of a T cell receptor with high affinity for peptide/MHC. Proc Natl Acad Sci U S A *97*, 5387-5392.

Hombach, A., Muche, J. M., Gerken, M., Gellrich, S., Heuser, C., Pohl, C., Sterry, W., and Abken, H. (2001). T cells engrafted with a recombinant anti-CD30 receptor target autologous CD30(+) cutaneous lymphoma cells. Gene Ther *8*, 891-895.

Hombach, A., Schneider, C., Sent, D., Koch, D., Willemsen, R. A., Diehl, V., Kruis, W., Bolhuis, R. L., Pohl, C., and Abken, H. (2000). An entirely humanized CD3 zeta-chain signaling receptor that directs peripheral blood T cells to specific lysis of carcinoembryonic antigen-positive tumor cells. Int J Cancer *88*, 115-120.

Hu, J., and Dunbar, C. E. (2002). Update on hematopoietic stem cell gene transfer using non-human primate models. Curr Opin Mol Ther *4*, 482-490.

Hung, K., Hayashi, R., Lafond-Walker, A., Lowenstein, C., Pardoll, D., and Levitsky, H. (1998). The central role of CD4(+) T cells in the antitumor immune response. J Exp Med *188*, 2357-2368.

Hwu, P., Yang, J. C., Cowherd, R., Treisman, J., Shafer, G. E., Eshhar, Z., and Rosenberg, S. A. (1995). In vivo antitumor activity of T cells redirected with chimeric antibody T cell receptor genes. Cancer Res *55*, 3369-3373.

Irvine, K. R., Parkhurst, M. R., Shulman, E. P., Tupesis, J. P., Custer, M., Touloukian, C. E., Robbins, P. F., Yafal, A. G., Greenhalgh, P., Sutmuller, R. P. M., *et al.* (1999). Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens. Cancer Res *59*, 2536-2540.

Jenner, E. (1798). Sampson Low, Soho, London).

Kaiser, J. (2003). Gene therapy: Seeking the cause of induced leukemias in X-SCID trial. Science *299*, 495-495.

Kamelreid, S., and Dick, J. E. (1988). Engraftment of immune-deficient mice with human hematopoietic stem cells. Science *242*, 1706-1709.

Kershaw, M. H., Westwood, J. A., and Hwu, P. (2002). Dual-specific T cells combine proliferation and antitumor activity. Nat Biotechnol *20*, 1221-1227.

Kershaw, M. H., Westwood, J. A., Zhu, Z. P., Witte, L., Libutti, S. K., and Hwu, P. (2000). Generation of gene-modified T cells reactive against the angiogenic kinase insert domain-containing receptor (KDR) found on tumor vasculature. Hum Gene Ther *11*, 2445-2452.

Kessels, H., van den Boom, M. D., Spits, H., Hooijberg, E., and Schumacher, T. N. M. (2000). Changing T cell specificity by retroviral T cell receptor display. Proc Natl Acad Sci U S A *97*, 14578-14583.

Kessels, H., Wolkers, M. C., van den Boom, M. D., van der Valk, M. A., and Schumacher, T. N. M. (2001). Immunotherapy through TCR gene transfer. Nat Immunol *2*, 957-961.

Khong, H. T., and Restifo, N. P. (2002). Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat Immunol *3*, 999-1005.

Kim, J. W., Closs, E. I., Albritton, L. M., and Cunningham, J. M. (1991). Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. Nature *352*, 725-728.

Koch, F., Stanzl, U., Jennewein, P., Janke, K., Heufler, C., Kampgen, E., Romani, N., and Schuler, G. (1996). High level IL-12 production by murine dendritic cells: Upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. J Exp Med *184*, 741-746.

Kohn, D. B. (1999). Gene therapy using hematopoietic stem cells. Curr Opin Mol Ther *1*, 437-442.

Kollet, O., Peled, A., Byk, T., Ben-Hur, H., Greiner, D., Shultz, L., and Lapidot, T. (2000). beta 2 microglobulin-deficient (B2m(null)) NOD/SCID mice are excellent recipients for studying human stem cell function. Blood *95*, 3102-3105.

Kondo, M., Wagers, A. J., Manz, M. G., Prohaska, S. S., Scherer, D. C., Beilhack, G. E., Shizuru, J. A., and Weissman, I. L. (2003). Biology of hematopoietic stem cells and progenitors: Implications for clinical application. Annu Rev Immunol *21*, 759-806.

Krause, A., Guo, H. F., Latouche, J. B., Tan, C. W., Cheung, N. K. V., and Sadelain, M. (1998). Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. J Exp Med *188*, 619-626.

Kreitman, R. J., Wilson, W., Waldmann, T. A., White, J. D., Pearson, D., Dyer, V., Margulies, I., Stetler-Stevenson, M. A., and Pastan, I. (1998). Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. Blood *92*, 413A-413A.

Krug, A., Rothenfusser, S., Selinger, S., Bock, C., Kerkmann, M., Battiany, J., Sarris, A., Giese, T., Speiser, D., Endres, S., and Hartmann, G. (2003). CpG-A oligonucleotides induce a monocyte-derived dendritic cell-like phenotype that preferentially activates CD8 T cells. J Immunol *170*, 3468-3477.

Lapidot, T., Pflumio, F., Doedens, M., Murdoch, B., Williams, D. E., and Dick, J. E. (1992). Cytokine Stimulation of Multilineage Hematopoiesis from Immature Human-Cells Engrafted in Scid Mice. Science *255*, 1137-1141.

Lenardo, M. J. (1996). Fas and the art of lymphocyte maintenance. J Exp Med *183*, 721-724.

Leurs, C., Jansen, M., Pollok, K. E., Heinkelein, M., Schmidt, M., Wissler, M., Lindemann, D., Von Kalle, C., Rethwilm, A., Williams, D. A., and Hanenberg, H. (2003). Comparison of three retroviral vector systems for transduction of nonobese diabetic/severe combined immunodeficiency mice repopulating human CD34(+) cord blood cells. Hum Gene Ther *14*, 509-519.

Levitsky, H. I., Lazenby, A., Hayashi, R. J., and Pardoll, D. M. (1994). In vivo priming of 2 distinct antitumor effector populations: the role of MHC class I expression. J Exp Med *179*, 1215-1224.

Lewis, P., Hensel, M., and Emerman, M. (1992). Human immunodeficiency virus infection of cells arrested in the cell cycle. Embo J *11*, 3053-3058.

Li, Z. X., Dullmann, J., Schiedlmeier, B., Schmidt, M., von Kalle, C., Meyer, J., Forster, M., Stocking, C., Wahlers, A., Frank, O.*, et al.* (2002). Murine leukemia induced by retroviral gene marking. Science *296*, 497-497.

Lo, M., Bloom, M. L., Imada, K., Berg, M., Bollenbacher, J. M., Bloom, E. T., Kelsall, B. L., and Leonard, W. J. (1999). Restoration of lymphoid populations in a murine model of X- linked severe combined immunodeficiency by a gene-therapy approach. Blood *94*, 3027-3036.

Lo-Man, R., Rueda, P., Sedlik, C., Deriaud, E., Casal, I., and Leclerc, C. (1998). A recombinant virus-like particle system derived from parvovirus as an efficient antigen carrier to elicit a polarized Th1 immune response without adjuvant. Eur J Immunol *28*, 1401-1407.

Lubin, I., Faktorowich, Y., Lapidot, T., Gan, Y., Eshhar, Z., Gazit, E., Levite, M., and Reisner, Y. (1991). Engraftment and development of human T cells and B cells in mice after bone marrow transplantation. Science *252*, 427-431.

Maher, J., Brentjens, R. J., Gunset, G., Riviere, I., and Sadelain, M. (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCR zeta/CD28 receptor. Nat Biotechnol *20*, 70-75.

Malek, T. R., Porter, B. O., Codias, E. K., Scibelli, P., and Yu, A. X. (2000). Normal lymphoid homeostasis and lack of lethal autoimmunity in mice containing mature T cells with severely impaired IL-2 receptors. J Immunol *164*, 2905-2914.

Malek, T. R., Yu, A. X., Vincek, V., Scibelli, P., and Kong, L. (2002). CD4 regulatory T cells prevent lethal autoimmunity in IL-2R beta-deficient mice: implications for the nonredundant function of IL-2. Immunity *17*, 167-178.

Mardiney, M., Jackson, S. H., Spratt, S. K., Li, F., Holland, S. M., and Malech, H. L. (1997). Enhanced host defense after gene transfer in the murine p47(phox)-deficient model of chronic granulomatous disease. Blood *89*, 2268-2275.

Marshall, E. (2003). Gene therapy: Second child in French trial is found to have leukemia. Science *299*, 320-320.

May, C., Rivella, S., Callegari, J., Heller, G., Gaensler, K. M. L., Luzzatto, L., and Sadelain, M. (2000). Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature *406*, 82-86.

McGuinness, R. P., Ge, Y., Patel, S. D., Kashmiri, S. V. S., Lee, H. S., Hand, P. H., Schlom, J., Finer, M. H., and McArthur, J. G. (1999). Anti-tumor activity of human T cells expressing the CC49-zeta chimeric immune receptor. Hum Gene Ther *10*, 165-173.

McHugh, R. S., and Shevach, E. M. (2002). The role of suppressor T cells in regulation of immune responses. J Allergy Clin Immunol *110*, 693-702.

McLaughlin, P. (1999). Anti-CD20 (rituximab) in follicular lymphoma. Eur J Cancer *35*, S51-S51.

McLaughlin, P., Grillo-Lopez, A. J., Link, B. K., Levy, R., Czuczman, M. S., Williams, M. E., Heyman, M. R., Bence-Bruckler, I., White, C. A., Cabanillas, F.*, et al.* (1998a). Rituximab chimeric Anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four- dose treatment program. J Clin Oncol *16*, 2825-2833.

McLaughlin, P., Grillo-Lopez, A. J., Maloney, D. G., Link, B. K., Levy, R., Czuczman, M. S., Cabanillas, F., Dallaire, B. K., and White, C. A. (1998b). Efficacy controls and long-term follow-up

of patients (pts) treated with rituximab for relapsed or refractory, low-grade or follicular (R-LG/F) NHL. Blood *92*, 414A-415A.

McLaughlin, P., White, C. A., Grillo-Lopez, A. J., and Maloney, D. G. (1998c). Clinical status and optimal use of rituximab for B-cell lymphomas. Oncology-NY *12*, 1763-1769.

Medzhitov, R. (2001). CpG DNA: security code for host defense. Nat Immunol *2*, 15-16.

Mendoza, G. J. A., Seidel, N. E., Otsu, M., Anderson, S. M., Simon-Stoos, K., Herrera, A., Hoogstraten-Miller, S., Malech, H. L., Candotti, F., Puck, J. M., and Bodine, D. M. (2001). Comparison of five retrovirus vectors containing the human IL-2 receptor gamma chain gene for their ability to restore T and B lymphocytes in the X-linked severe combined immunodeficiency mouse model. Mol Ther *3*, 565-573.

Micklem, H. S., Ogden, D. A., Evans, E. P., Ford, C. E., and Papworth, D. S. (1972). Competitive in vivo proliferation of fetal and adult hemopoietic cells in lethally irradiated mice. J Cell Physiol *79*, 293-&.

Miller, A. D. (1992a). Human gene therapy comes of age. Nature *357*, 455-460.

Miller, A. D. (1992b). Retroviral vectors. Curr Top Microbiol Immunol *158*, 1-24.

Miller, A. D. (2003). Identification of Hyal2 as the cell-surface receptor for jaagsiekte sheep retrovirus and ovine nasal adenocarcinoma virus. In Jaagsiekte Sheep Retrovirus and Lung Cancer, pp. 179-199.

Miller, A. D., Miller, D. G., Garcia, J. V., and Lynch, C. M. (1993). Use of retroviral vectors for gene transfer and expression. Method Enzymol *217*, 581-599.

Miller, D. G., Edwards, R. H., and Miller, A. D. (1994). Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for Gibbon APE leukemia virus. Proc Natl Acad Sci U S A *91*, 78-82.

Miller, J. L., Donahue, R. E., Sellers, S. E., Samulski, R. J., Young, N. S., and Nienhuis, A. W. (1995). Recombinant adenoassociated virus (Raav)-mediated expression of a human gamma-globin gene in human progenitor-derived erythroid cells. Proc Natl Acad Sci U S A *92*, 646-646.

Molineux, G., McCrea, C., Yan, X. Q., Kerzic, P., and McNiece, I. (1997). Flt-3 ligand synergizes with granulocyte colony-stimulating factor to increase neutrophil numbers and to mobilize peripheral blood stem cells with long-term repopulating potential. Blood *89*, 3998-4004.

Mucenski, M. L., Taylor, B. A., Ihle, J. N., Hartley, J. W., Morse, H. C., Jenkins, N. A., and Copeland, N. G. (1988). Identification of a common ecotropic viral integration site, EVI-1, in the DNA of akxd murine myeloid tumors. Mol Cell Biol *8*, 301-308.

Mulligan, R. C. (1993). The basic science of gene therapy. Science *260*, 926-932.

Nakano, T., Waki, N., Asai, H., and Kitamura, Y. (1989). Lymphoid differentiation of the hematopoietic stem cell that reconstitutes total erythropoiesis of a genetically anemic W/Wv mouse. Blood *73*, 1175-1179.

Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M., and Trono, D. (1996). In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science *272*, 263-267.

Neben, S., Marcus, K., and Mauch, P. (1993). Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and or Granulocyte Colony-Stimulating Factor. Blood *81*, 1960-1967.

Neipp, M., Zorina, T., Domenick, M. A., Exner, B. G., and Ildstad, S. T. (1998). Effect of FLT3 ligand and granulocyte colony-stimulating factor on expansion and mobilization of facilitating cells and hematopoietic stem cells in mice: Kinetics and repopulating potential. Blood *92*, 3177-3188.

Nelson, B. H. (2004). IL-2, Regulatory T Cells, and Tolerance. J Immunol *172*, 3983-3988.

Nestle, F. O., Banchereau, J., and Hart, D. (2001). Dendritic cells: On the move from bench to bedside. Nature Medicine *7*, 761-765.

Niederman, T. M. J., Ghogawala, Z., Carter, B. S., Tompkins, H. S., Russell, M. M., and Mulligan, R. C. (2002). Antitumor activity of cytotoxic T lymphocytes engineered to target vascular endothelial growth factor receptors. Proc Natl Acad Sci U S A *99*, 7009-7014.

Nolan, K. F., Yun, C. O., Akamatsu, Y., Murphy, J. C., Leung, S. O., Beecham, E. J., and Junghans, R. P. (1999). Bypassing immunization: Optimized design of "designer T cells" against carcinoembryonic antigen (CEA)-expressing tumors, and lack of suppression by soluble CEA. Clin Cancer Res *5*, 3928-3941.

Nolta, J. A., Hanley, M. B., and Kohn, D. B. (1994). Sustained human hematopoiesis in immunodeficient mice by cotransplantation of marrow stroma expressing human Interleukin-3: Analysis of gene transduction of long lived progenitors. Blood *83*, 3041-3051.

Nouri-Shirazi, M., Banchereau, J., Bell, D., Burkeholder, S., Kraus, E. T., Davoust, J., and Palucka, K. A. (2000). Dendritic cells capture killed tumor cells and present their antigens to elicit tumor-specific immune responses. J Immunol *165*, 3797-3803.

Ochsenbein, A. F. (2002). Principles of tumor immunosurveillance and implications for immunotherapy. Cancer Gene Ther *9*, 1043-1055.

Ochsenbein, A. F., Sierro, S., Odermatt, B., Pericin, M., Karrer, U., Hermans, J., Hemmi, S., Hengartner, H., and Zinkernagel, R. M. (2001). Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. Nature *411*, 1058-1064.

O'Hagan, D. T., Singh, M., and Gupta, R. K. (1998). Poly(lactide-co-glycolide) microparticles for the development of single-dose controlled-release vaccines. Adv Drug Deliv Rev *32*, 225-246.

Onodera, M., Nelson, D. M., Yachie, A., Jagadeesh, G. J., Bunnell, B. A., Morgan, R. A., and Blaese, R. M. (1998). Development of improved adenosine deaminase retroviral vectors. J Virol *72*, 1769-1774.

Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S. M., Li, B. S., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D. M., *et al.* (2001). Bone marrow cells regenerate infarcted myocardium. Nature *410*, 701-705.

Ory, D. S., Neugeboren, B. A., and Mulligan, R. C. (1996). A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes. Proc Natl Acad Sci U S A *93*, 11400-11406.

Ossendorp, F., Mengede, E., Camps, M., Filius, R., and Melief, C. J. M. (1998). Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. J Exp Med *187*, 693-702.

Ostrandrosenberg, S. (1994). Tumor-Immunotherapy: the tumor cell as an antigen presenting cell. Curr Opin Immunol *6*, 722-727.

Otsu, M., Sugamura, K., and Candotti, F. (2000). In vivo competitive studies between normal and common gamma chain-defective bone marrow cells: Implications for gene therapy. Hum Gene Ther *11*, 2051-2056.

Otsu, M., Sugamura, K., and Candotti, F. (2001). Lack of dominant-negative effects of a truncated gamma c on retroviral-mediated gene correction of immunodeficient mice. Blood *97*, 1618-1624.

Pan, Z. K., Ikonomidis, G., Pardoll, D., and Paterson, Y. (1995). Regression of established tumors in mice mediated by the oral administration of a recombinant Listeria-monocytogenes vaccine. Cancer Res *55*, 4776-4779.

Pardoll, D. (2002a). T cells take aim at cancer. Proc Natl Acad Sci U S A *99*, 15840-15842.

Pardoll, D. M. (2002b). Spinning molecular immunology into successful immunotherapy. Nat Rev Immunol *2*, 227-238.

Pardoll, D. M., and Topalian, S. L. (1998). The role of CD4(+) T cell responses in antitumor immunity. Curr Opin Immunol *10*, 588-594.

Parker, L. L., Do, M. T., Westwood, J. A., Wunderlich, J. R., Dudley, M. E., Rosenberg, S. A., and Hwu, P. (2000). Expansion and characterization of T cells transduced with a chimeric receptor against ovarian cancer. Hum Gene Ther *11*, 2377-2387.

Parkhurst, M. R., Salgaller, M. L., Southwood, S., Robbins, P. F., Sette, A., Rosenberg, S. A., and Kawakami, Y. (1996). Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201-binding residues. J Immunol *157*, 2539-2548.

Patel, S. D., Ge, Y., Moskalenko, M., and McArthur, J. G. (2000). Anti-tumor CC49-zeta CD4 T cells possess both cytolytic and helper functions. J Immunother *23*, 661-668.

Pawliuk, R., Eaves, C. J., and Humphries, R. K. (1996). High level multilineage reconstitution of mice with sustained expression of a transduced gene encoding a cell surface antigen. Blood *88*, 2738-2738.

Pawliuk, R., Eaves, C. J., and Humphries, R. K. (1997). Sustained high-level reconstitution of the hematopoietic system by preselected hematopoietic cells expressing a transduced cell-surface antigen. Hum Gene Ther *8*, 1595-1604.

Pawliuk, R., Westerman, K. A., Fabry, M. E., Payen, E., Tighe, R., Bouhassira, E. E., Acharya, S. A., Ellis, J., London, I. M., Eaves, C. J., *et al.* (2001). Correction of sickle cell disease in transgenic mouse models by gene therapy. Science *294*, 2368-2371.

Poeschla, E. M. (2003). Non-primate lentiviral vectors. Curr Opin Mol Ther *5*, 529-540.

Pogue, R. R., Eron, J., Frelinger, J. A., and Matsui, M. (1995). Amino terminal alteration of the HLA-A-Asterisk-0201-restricted human immunodeficiency virus Pol peptide increases complex stability and in vitro immunogenicity. Proc Natl Acad Sci U S A *92*, 8166-8170.

Ponnazhagan, S., Weigel, K. A., Raikwar, S. P., Mukherjee, P., Yoder, M. C., and Srivastava, A. (1998). Recombinant human parvovirus B19 vectors: Erythroid cell- specific delivery and expression of transduced genes. J Virol *72*, 5224-5230.

Prevost-Blondel, A., Zimmermann, C., Stemmer, C., Kulmburg, P., Rosenthal, F. M., and Pircher, H. (1998). Tumor-infiltrating lymphocytes exhibiting high ex vivo cytolytic activity fail to prevent murine melanoma tumor growth in vivo. J Immunol *161*, 2187-2194.

Pulaski, B. A., McAdam, A. J., Hutter, E. K., Biggar, S., Lord, E. M., and Frelinger, J. G. (1993). Interleukin-3 enhances development of tumor reactive cytotoxic cells by a CD4 dependent mechanism. Cancer Res *53*, 2112-2117.

Qin, Z. H., Richter, G., Schuler, T., Ibe, S., Cao, X. T., and Blankenstein, T. (1998). B cells inhibit induction of T cell-dependent tumor immunity. Nature Medicine *4*, 627-630.

Ravetch, J. V., and Clynes, R. A. (1998). Divergent roles for Fc receptors and complement in vivo. Annu Rev Immunol *16*, 421-432.

Ren-Heidenreich, L., Mordini, R., Hayman, G. T., Siebenlist, R., and LeFever, A. (2002). Comparison of the TCR zeta-chain with the FcR gamma-chain in chimeric TCR constructs for T cell activation and apoptosis. Cancer Immunol Immunother *51*, 417-423.

Renkvist, N., Castelli, C., Robbins, P. F., and Parmiani, G. (2001). A listing of human tumor antigens recognized by T cells. Cancer Immunol Immunother *50*, 3-15.

Ridge, J. P., Di Rosa, F., and Matzinger, P. (1998). A conditioned dendritic cell can be a temporal bridge between a CD4(+) T-helper and a T-killer cell. Nature *393*, 474-478.

Robbins, P. B., Yu, X. J., Skelton, D. M., Pepper, K. A., Wasserman, R. M., Zhu, L., and Kohn, D. B. (1997). Increased probability of expression from modified retroviral vectors in embryonal stem cells and embryonal carcinoma cells. J Virol *71*, 9466-9474.

Rooney, C. M., Smith, C. A., Ng, C. Y., Loftin, S. K., Li, C., Krance, R. A., Brenner, M. K., and Heslop, H. (1995). Genetically marked virus-specific CTLs to control EBV-related lymphoproliferation. Exp Hematol *23*, 851-851.

Rosenberg, S. A., Yang, J. C., Schwartzentruber, D. J., Hwu, P., Marincola, F. M., Topalian, S. L., Restifo, N. P., Dudley, M. E., Schwarz, S. L., Spiess, P. J.*, et al.* (1998). Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nature Medicine *4*, 321-327.

Rosenberg, S. A., Yang, J. C., Topalian, S. L., Schwartzentruber, D. J., Weber, J. S., Parkinson, D. R., Seipp, C. A., Einhorn, J. H., and White, D. E. (1994). Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus Interleukin-2. JAMA-J Am Med Assoc *271*, 907-913.

Russell, N. H., Gratwohl, A., and Schmitz, N. (1998). Developments in allogeneic peripheral blood progenitor cell transplantation. Br J Haematol *103*, 594-600.

Sadelain, M., Riviere, I., and Brentjens, R. (2003). Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer *3*, 35-45.

Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C., and Horak, I. (1993). Ulcerative Colitis-like disease in mice with a disrupted Interleukin-2 gene. Cell *75*, 253-261.

Sakaguchi, S., Sakaguchi, N., Shimizu, J., Yamazaki, S., Sakihama, T., Itoh, M., Kuniyasu, Y., Nomura, T., Toda, M., and Takahashi, T. (2001). Immunologic tolerance maintained by CD25(+) CD4(+) regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev *182*, 18-32.

Salgaller, M. L., and Lodge, P. A. (1998). Use of cellular and cytokine adjuvants in the immunotherapy of cancer. J Surg Oncol *68*, 122-138.

Sarobe, P., Pendleton, C. D., Akatsuka, T., Lau, D., Engelhard, V. H., Feinstone, S. M., and Berzofsky, J. A. (1998). Enhanced in vitro potency and in vivo immunogenicity of a CTL epitope from hepatitis C virus core protein following amino acid replacement at secondary HLA-A2.1 binding positions. J Clin Invest *102*, 1239-1248.

Schlegel, R., Tralka, T. S., Willingham, M. C., and Pastan, I. (1983). Inhibition of VSV binding and infectivity by phosphatidylserine: Is phosphatidylserine a VSV-binding site. Cell *32*, 639-646.

Schluns, K. S., and Lefrancois, L. (2003). Cytokine control of memory T-cell development and survival. Nat Rev Immounol *3*, 269-279.

Schoenberger, S. P., Toes, R. E. M., van der Voort, E. I. H., Offringa, R., and Melief, C. J. M. (1998). T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature *393*, 480-483.

Shevach, E. M. (2002). CD4(+)CD25(+) suppressor T cells: More questions than answers. Nat Rev Immunol *2*, 389-400.

Shevach, E. M. (2003). Control of T-cell responses by regulatory/suppressor T cells. Exp Dermatol *12*, 913-914.

Shimizu, J., Yamazaki, S., and Sakaguchi, S. (1999). Induction of tumor immunity by removing CD25(+)CD4(+) T cells: A common basis between tumor immunity and autoimmunity. J Immunol *163*, 5211-5218.

Shimizu, Y., Ogawa, M., Kobayashi, M., Almeida-Porada, G., and Zanjani, E. D. (1998). Engraftment of cultured human hematopoietic cells in sheep. Blood *91*, 3688-3692.

Shinde, S., Wu, Y., Guo, Y., Niu, Q. T., Xu, J. C., Grewal, I. S., Flavell, R., and Liu, Y. (1996). CD40L is important for induction of, but not response to, costimulatory activity (ICAM-1) as the second costimulatory molecule rapidly up-regulated by CD40L. J Immunol *157*, 2764-2768.

Shultz, L. D. (1991). Immunological mutants of the mouse. American Journal of Anatomy *191*, 303-311.

Shultz, L. D., Schweitzer, P. A., Christianson, S. W., Gott, B., Schweitzer, I. B., Tennent, B., McKenna, S., Mobraaten, L., Rajan, T. V., Greiner, D. L., and Leiter, E. H. (1995). Multiple defects in innate and adaptive immunological function in NOD/LTSZ-SCID mice. J Immunol *154*, 180-191.

Slansky, J. E., Rattis, F. M., Boyd, L. F., Fahmy, T., Jaffee, E. M., Schneck, J. P., Margulies, D. H., and Pardoll, D. M. (2000). Enhanced antigen-specific antitumor immunity with altered peptide ligands that stabilize the MHC-peptide-TCR complex. Immunity *13*, 529-538.

Srivastava, P. (2002). Interaction of heat shock proteins with peptides and antigen presenting cells: Chaperoning of the innate and adaptive immune responses. Annu Rev Immunol *20*, 395-425.

Srour, E. F., Zanjani, E. D., Brandt, J. E., Cornetta, K., Flake, A. W., Hedrick, M., Leemhuis, T., and Hoffman, R. (1992a). Detection of human stem cells in chimeric sheep transplanted with adult human CD34+ HLA-DR bone marrow cells. Exp Hematol *20*, 746-746.

Srour, E. F., Zanjani, E. D., Brandt, J. E., Leemhuis, T., Briddell, R. A., Heerema, N. A., and Hoffman, R. (1992b). Sustained human hematopoiesis in sheep transplanted Inutero during early gestation with fractionated adult human bone marrow cells. Blood *79*, 1404-1412.

Srour, E. F., Zanjani, E. D., Cornetta, K., Traycoff, C. M., Flake, A. W., Hedrick, M., Brandt, J. E., Leemhuis, T., and Hoffman, R. (1993). Persistence of human multilineage, self-renewing lymphohematopoietic stem cells in chimeric sheep. Blood *82*, 3333-3342.

Stanislawski, T., Voss, R. H., Lotz, C., Sadovnikova, E., Willemsen, R. A., Kuball, J., Ruppert, T., Bolhuis, R. L. H., Melief, C. J., Huber, C.*, et al.* (2001). Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. Nat Immunol *2*, 962-970.

Staveley-O'Carroll, K., Sotomayor, E., Montgomery, J., Borrello, I., Hwang, L., Fein, S., Pardoll, D., and Levitsky, H. (1998). Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. Proc Natl Acad Sci U S A *95*, 1178-1183.

Sutmuller, R. P. M., van Dulvenvoorde, L. M., van Elsas, A., Schumacher, T. N. M., Wildenberg, M. E., Allison, J. P., Toes, R. E. M., Offringa, R., and Melief, C. J. M. (2001). Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med *194*, 823-832.

Sutton, R. E., Wu, H. T. M., Rigg, R., Bohnlein, E., and Brown, P. O. (1998). Human immunodeficiency virus type 1 vectors efficiently transduce human hematopoietic stem cells. J Virol *72*, 5781-5788.

Suzuki, H., Kundig, T. M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., Schmits, R., Simard, J. J. L., Ohashi, P. S., Griesser, H.*, et al.* (1995). Deregulated T-cell activation and autoimmunity in mice lacking Interleukin-2 receptor-beta. Science *268*, 1472-1476.

Suzuki, T., Shen, H. F., Akagi, K., Morse, H. C., Malley, J. D., Naiman, D. Q., Jenkins, N. A., and Copeland, N. G. (2002). New genes involved in cancer identified by retroviral tagging. Nature Genet *32*, 166-174.

Sykes, M., Sachs, D. H., Nienhuis, A. W., Pearson, D. A., Moulton, A. D., and Bodine, D. M. (1993). Specific prolongation of skin-graft survival following retroviral transduction of bone marrow with an allogeneic major histocompatibility complex gene. Transplantation *55*, 197-202.

Takahashi, H., Takeshita, T., Morein, B., Putney, S., Germain, R. N., and Berzofsky, J. A. (1990). Induction of CD8 cytotoxic T cells by immunization with purified HIV-1 envelope protein in Iscoms. Nature *344*, 873-875.

Thomas, E. D., Storb, R., Clift, R. A., Fefer, A., Johnson, F. L., Neiman, P. E., Lerner, K. G., Glucksberg, H., and Buckner, C. D. (1975a). Bone marrow transplantation 1. N Engl J Med *292*, 832-843.

Thomas, E. D., Storb, R., Clift, R. A., Fefer, A., Johnson, F. L., Neiman, P. E., Lerner, K. G., Glucksberg, H., and Buckner, C. D. (1975b). Bone marrow transplantation 2. N Engl J Med *292*, 895-902.

Van Parijs, L., Refaeli, Y., Lord, J. D., Nelson, B. H., Abbas, A. K., and Baltimore, D. (1999). Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. Immunity *11*, 281-288.

Varmus, H. (1988). Retroviruses. Science *240*, 1427-1435.

Varmus, H. E. (1982). Form and function of retroviral proviruses. Science *216*, 812-820.

Waldmann, T. A. (2003). Immunotherapy: past, present and future. Nat Med *9*, 269-277.

Walsh, C. E., Grompe, M., Young, N. S., Nienhuis, A. W., and Liu, J. M. (1993). A functionally active retrovirus vector for gene therapy in Fanconis Anemia Type C. Blood *82*, A176-A176.

Walter, E. A., Greenberg, P. D., Gilbert, M. J., Finch, R. J., Watanabe, K. S., Thomas, E. D., and Riddell, S. R. (1995). Reconstitution of cellular immunity against Cytomegalovirus in recipients of allogeneic bone marrow by transfer of T cell clones from the donor. N Engl J Med *333*, 1038-1044.

Wang, G., Chopra, R. K., Royal, R. E., Yang, J. C., Rosenberg, S. A., and Hwu, P. (1998). A T cell-independent antitumor response in mice with bone marrow cells retrovirally transduced with an antibody Fc-gamma chain chimeric receptor gene recognizing a human ovarian cancer antigen. Nature Medicine *4*, 168-172.

Warren, E. H., Greenberg, P. D., and Riddell, S. R. (1998). Cytotoxic T-lymphocyte-defined human minor histocompatibility antigens with a restricted tissue distribution. Blood *91*, 2197-2207.

Weissman, I. L. (2002). The road ended up at stem cells. Immunol Rev *185*, 159-174.

Weissman, I. L., Anderson, D. J., and Gage, F. (2001). Stem and progenitor cells: Origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol *17*, 387-403.

Whiteside, T. L., Gambotto, A., Albers, A., Stanson, J., and Cohen, E. P. (2002). Human tumor-derived genomic DNA transduced into a recipient cell induces tumor-specific immune responses ex vivo. Proc Natl Acad Sci U S A *99*, 9415-9420.

Wick, M., Dubey, P., Koeppen, H., Siegel, C. T., Fields, P. E., Chen, L. P., Bluestone, J. A., and Schreiber, H. (1997). Antigenic cancer cells grow progressively in immune hosts without evidence for T cell exhaustion or systemic anergy. J Exp Med *186*, 229-238.

Willerford, D. M., Chen, J. Z., Ferry, J. A., Davidson, L., Ma, A., and Alt, F. W. (1995). Interleukin-2 receptor-alpha chain regulates the size and content of the peripheral lymphoid compartment. Immunity *3*, 521-530.

Williams, D. A. (1990). Expression of introduced genetic sequences in hematopoietic cells following retroviral-dediated gene transfer. Hum Gene Ther *1*, 229-239.

Williams, D. A., and Smith, F. O. (2000). Progress in the use of gene transfer methods to treat genetic blood diseases. Hum Gene Ther *11*, 2059-2066.

Wilson, C. A., Ng, T. H., and Miller, A. E. (1997). Evaluation of recommendations for replication-competent retrovirus testing associated with use of retroviral vectors. Hum Gene Ther *8*, 869-874.

Wilson, J. M., Danos, O., Grossman, M., Raulet, D. H., and Mulligan, R. C. (1990). Expression of human adenosine deaminase in mice reconstituted with retrovirus-transduced hematopoietic stem cells. Proc Natl Acad Sci U S A *87*, 439-443.

Witzig, T. E., Gordon, L. I., Cabanillas, F., Czuczman, M. S., Emmanouilides, C., Joyce, R., Pohlman, B. L., Bartlett, N. L., Wiseman, G. A., Padre, N., *et al.* (2002). Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab

immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol *20*, 2453-2463.

Wolfe, J. H., Sands, M. S., Barker, J. E., Gwynn, B., Rowe, L. B., Vogler, C. A., and Birkenmeier, E. H. (1992). Reversal of pathology in murine mucopolysaccharidosis type-VII by somatic cell gene transfer. Nature *360*, 749-753.

Yang, Y. P., Vanin, E. F., Whitt, M. A., Fornerod, M., Zwart, R., Schneiderman, R. D., Grosveld, G., and Nienhuis, A. W. (1995). Inducible, high level production of infectious murine leukemia retroviral vector particles pseudotyped with vesicular stomatitis virus G envelope protein. Hum Gene Ther *6*, 1203-1213.

Yee, C., Thompson, J. A., Byrd, D., Riddell, S. R., Roche, P., Celis, E., and Greenberg, P. D. (2002). Adoptive T cell therapy using antigen-specific CD8(+) T cell clones for the treatment of patients with metastatic melanoma: In vivo persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci U S A *99*, 16168-16173.

Yee, J. K., Miyanohara, A., Laporte, P., Bouic, K., Burns, J. C., and Friedmann, T. (1994). A general method for the generation of high titer, pantropic retroviral vectors - highly efficient infection of primary hepatocytes. Proc Natl Acad Sci U S A *91*, 9564-9568.

Yun, C. O., Nolan, K. F., Beecham, E. J., Reisfeld, R. A., and Junghans, R. P. (2000). Targeting of T lymphocytes to melanoma cells through chimeric anti-GD3 immunoglobulin T-cell receptors. Neoplasia *2*, 449-459.

Zanjani, E. D. (2000). The human sheep xenograft model for the study of the in vivo potential of human HSC and in utero gene transfer. Stem Cells *18*, 151-151.

Zanjani, E. D., Almeidaporada, G., and Flake, A. W. (1995a). Retention and multilineage expression of human hematopoietic stem cells in human-sheep chimeras. Stem Cells *13*, 101-111.

Zanjani, E. D., AlmeidaPorada, G., and Flake, A. W. (1996). The human/sheep xenograft model: A large animal model of human hematopoiesis. Int J Hematol *63*, 179-192.

Zanjani, E. D., Srour, E. F., and Hoffman, R. (1995b). Retention of long term repopulating ability of Xenogeneic transplanted purified adult human bone marrow hematopoietic stem cells in sheep. J Lab Clin Med *126*, 24-28.

Zaremba, S., Barzaga, E., Zhu, M. Z., Soares, N., Tsang, K. Y., and Schlom, J. (1997). Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. Cancer Res *57*, 4570-4577.