Review

Immunology of Stem Cells and Cancer Stem Cells

Xiao-Feng Yang[1, 2]

The capacity of pluri-potent stem cells to repair the tissues in which stem cells reside holds great promise in development of novel cell replacement therapeutics for treating chronic and degenerative diseases. However, numerous reports show that stem cell therapy, even in an autologous setting, triggers lymphocyte infiltration and inflammation. Therefore, an important question to be answered is how the host immune system responds to engrafted autologous stem cells or allogeneous stem cells. In this brief review, we summarize the progress in several related areas in this field, including some of our data, in four sections: (1) immunogenicity of stem cells; (2) strategies to inhibit immune rejection to allograft stem cells; (3) immune responses to cancer stem cells; and (4) mesenchymal stem cells in immune regulation. Improvement of our understanding on these and other aspects of immune system-stem cell interplay would greatly facilitate the development of stem cell-based therapeutics for regenerative purposes. *Cellular & Molecular Immunology*. 2007;4 (3):161-171.

Key Words: immunogenicity, stem cell therapy, cancer stem cells, stimulation-responsive splicing model

Introduction

As aging progresses, the regenerative power of pluri-potent stem cells for tissue repair is often inadequate to sustain normal tissue function (1). Consequently, the incidence of chronic and degenerative diseases including Parkinson's disease (2), Alzheimer's disease (2), diabetes (3), heart disease (4), leukemia (5), and others (6) has significantly increased in the United States. Over 125 million people suffer from at least one chronic disease and the related medical costs account for 78% of total medical expenses. In the next 20 years, the proportion of populations over 85 years of age in western countries is anticipated to quadruple to reach 157 millions, placing an unsustainable burden on society (7). The capacity of pluri-potent stem cells to repair the tissues in which stem cells reside has been demonstrated due to various technological advances, which hold great promise as novel cell replacement therapeutics for treating chronic and degenerative diseases (1). Therefore, the emerging strategies in regenerative medicine will be very important in coping with the challenges outlined above.

Stem cells are defined as clonogenic, self-renewing progenitor cells that can generate one or more specialized cell types. Embryonic stem cell (ESC) lines, established in mouse in 1981 (8, 9) and followed by the characterization of human ESC lines in 1998 (10), are derived from the inner cell mass of the blastocyst and are capable of generating all differentiated cell types in the body. To date, there are more than 300 human ESC lines, but only 22 human ESC lines are commercially available and registered with the NIH (http://stemcells.nih.gov/research/registry/) (6). Adult (postnatal) stem cells are still pluri-potent, but their differentiation ability is restricted to the cell types of a particular tissue, being responsible for organ regeneration. Two general categories of reserve precursor cells exist within the body and are involved in the maintenance and repair of tissue in adults: a) lineage-committed progenitor cells, and b) lineage-uncommitted pluri-potent stem cells. Dr. Young and his colleagues summarized a long list of common characteristics of lineage-committed progenitor stem cells (11). Progenitor stem cells (progenitor cells) may be committed to one or more specific tissue lineages, which can be further classified into unipotent, bipotent, tripotent, or multipotent, respectively (11). Each progenitor cell for a particular tissue lineage has a unique profile of cell surface cluster of differentiation (CD) markers (11). Progenitor cells conform to Hayflick's limit of 50-70 population doublings (12).

Primitive stem cells within the bone marrow niche (13) (hematopoietic stem cell, HSC) possess functional versatility broader than expected, which is termed trans-differentiation or stem cell plasticity. Stem cell plasticity describes the ability of adult stem cells to cross lineage barriers and to adopt the expression profiles and functional phenotypes of cells unique to other tissues (14) HSC, expressing markers of the hematopoietic lineage ($CD45^+$) and of hematopoietic

[1]Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, USA;

[2]Corresponding to: Dr. Xiao-Feng Yang, Department of Pharmacology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140, USA. Tel: +01-215-707-5985, Fax: +01-215-707-7068, E-mail: xfyang@temple.edu

Received Jun 14, 2007. Accepted Jun 21, 2007.

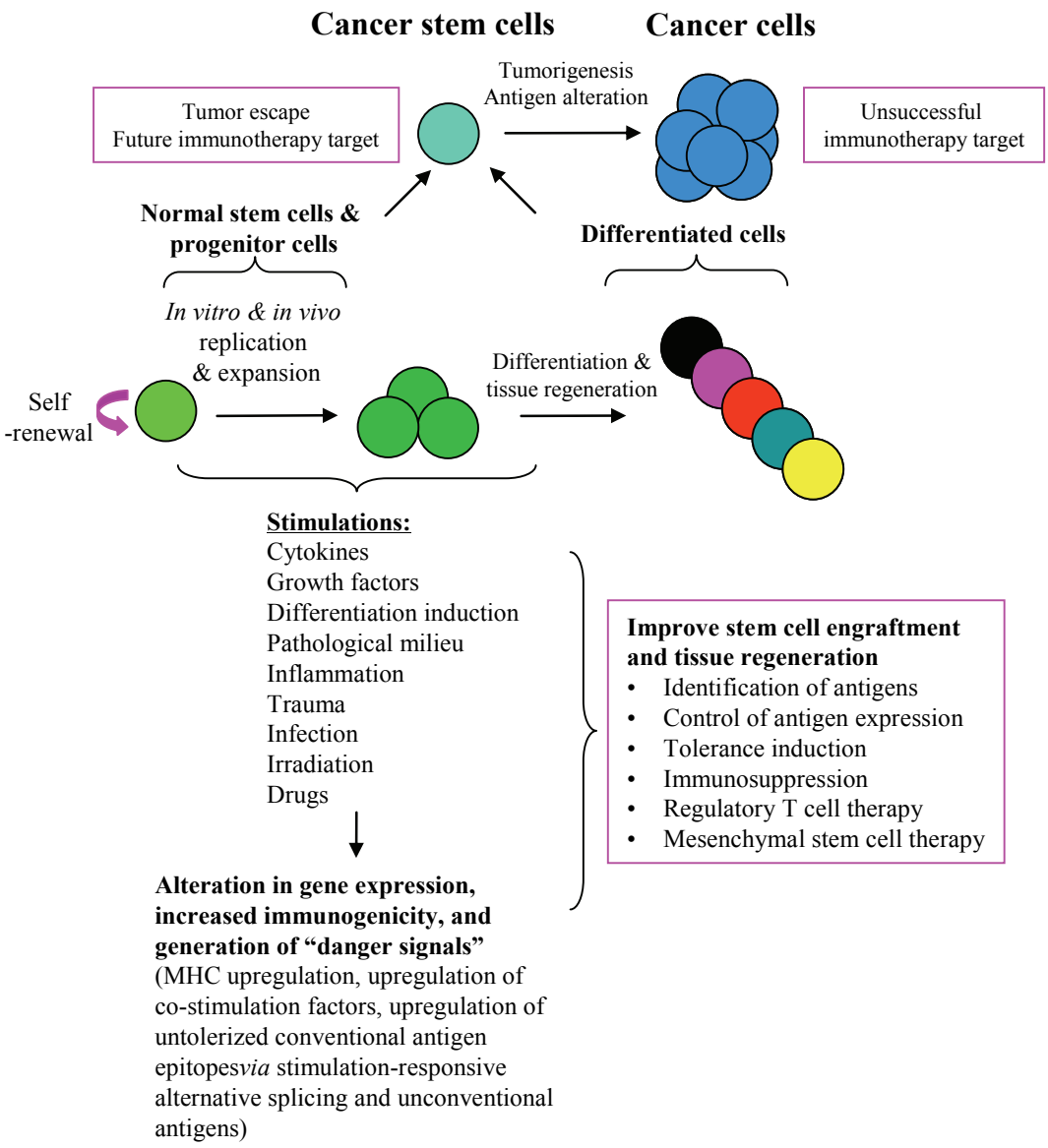

Figure 1. Several aspects of immunology of stem cells and cancer stem cells. To generate enough stem cells for regenerative medicine, stem cells with self-renewal properties need to experience the following two stages: a) *in vitro* & *in vivo* replication and expansion; and b) differentiation and tissue regeneration. During these processes, stem cells are stimulated by numerous factors, such as cytokines, growth factors, differentiation induction, pathological milieu, inflammation, trauma, infection, irradiation and drugs. Under the stimulation, gene expression in stem cells is altered, which leads to increased immunogenicity of stem cells and generation of "danger signals" by upregulation of MHC molecules, co-stimulation factors, and untolerized conventional antigen epitopes (*via* the mechanisms of stimulation-responsive alternative splicing and unconventional antigen expression). Therefore, to improve cell engraftment and tissue regeneration, several new strategies have been proposed including identification of stem cell antigens, control of aberrant antigen expression, tolerance induction, immunosuppression, regulatory T cell therapy, and mesenchymal stem cell therapy. In addition, normal stem cells and progenitor cells can be developed into cancer stem cells due to mutations. Cancer stem cells are believed to develop into, via tumorigenesis, cancer cells, latter of which have been unsuccessfully targeted by most of current immunotherapies. To enhance the efficacy of future cancer immunotherapy, both cancer stem cells and cancer cells need to be targeted.

stem cells ($CD34^+$, $CD133^+$, and $CD117^+$, Thy-1^{low}, but $CD10^-$, $CD14^-$, $CD15^-$, $CD16^-$, $CD19^-$, and $CD20^-$) (15, 16), are capable of genomic reprogramming upon exposure to a novel environment and give rise to other tissues such as liver, cardiac muscle, or brain (17, 18). Mouse HSCs' markers are CD117 (c-Kit)$^+$, Sca-1^+, and Thy-1^{low} but $B220^-$, $CD3^-$, $CD4^-$, $CD8^-$, Mac-1^-, Gr-1^-, and $Ter119^-$ (16). A defining property of murine hematopoietic stem cells (so-called side population) (19) is low fluorescence after staining with Hoechst 33342 and Rhodamine 123 (20). The HSCs in mice transplanted at the single cell level gave rise to lifelong hematopoiesis, including a steady state of 20 to 1 × 10^5 HSC and over 1 ×

10^9 blood cells produced daily (16). In addition to HSC, bone marrow also contains mesenchymal stem cells (MSCs), which have the capacity to proliferative extensively and form colonies of fibroblastic cells (defined as colony-forming units-fibroblastic; CFU-F) (21). Furthermore, discovery of cancer stem cells in leukemias and solid tumors (22) has added to the complexity of the stem cell field but stimulated great excitement for both stem cell and cancer biologists (23). Normal ESCs have been used in treating glycaemia in a mouse diabetes model (24), generating cardiomyocytes in dystrophic mice (25), improving cardiac function in post-infarcted rats (26), intervening in the progression of a rat model of Parkinson's disease (27). Due to the great potential of stem cells in development of novel therapy for chronic and degenerative diseases as well as cancers in autologous and allogeneic settings, several immunological aspects related to stem cell-based cell replacement therapy raise important concerns, as shown in Figure 1, which are the focus of this brief review.

Immunogenicity of stem cells

One of the important issues regarding stem cell therapy is whether stem cells can be used as immune privileged "BAND-AID" without elicitation of inflammation and immune responses (28). Transplantation of allogeneic undifferentiated murine ESCs in the heart cause cardiac teratomas, which are immunologically rejected after several weeks in association with increased inflammation and upregualtion of class I and II major histocompatibility complex (MHC) molecules (29). In addition, *in vivo* differentiated ESCs transplanted into ischemic myocardium elicit an accelerated immune response as compared with undifferentiated ESCs, suggesting ESC immunogenicity increases upon differentiation (30). Moreover, immune responses are not limited to transplanted ESCs. Transplantation of neural stem cells also induces immunological responses (31) and lymphocyte infiltration (32). Furthermore, transplantation of ESCs in the heart elicits infiltration of a few $CD3^+$ T cells even in the syngeneic mouse group, but not in the severe combined immunodeficiency disease (SCID) mouse group (33), the ESCs are not stealthy in the heart. In contrast to these findings, recent studies suggest some immune privilege is associated with human ESC-derived tissues (34-36). However, the adaptability of the immune system makes it unlikely that fully differentiated tissues will maintain their immune privilege and permanently evade immune rejection (5). Generally, an immune-privileged site, such as testis, does not express MHC class I or II molecules (37) and may express FasL to kill attacking lymphocytes (38). However, a recent report showed that the addition of Fas ligand (FasL) to healthy fetal MSC induces cell death by apoptosis (39), suggesting that stem cell death can be induced by attacking lymphocytes *via* a Fas/FasL mechanism. Human ES cells express low levels of MHC class I *in vitro* (40) in their undifferentiated state (41, 42) The MHC class I expression increases two to four-fold when the human ES cells are induced to differentiate to embryonic bodies, and an eight to ten-fold when induced to differentiate to teratomas (41). In contrast, other investigators observed MHC class I downregulation after differentiation induced with retinoic acid on Matrigel or in extended culture (42). MHC class I expression can be strongly upregulated after treatment of the ESCs with interferon-γ (43), a potent MHC expression-inducing proinflammatory cytokine known to be released during the course of immune responses (41, 42). Similarly, Bradley et al. reported that a four-fold expression of HLA class II molecules in human ESCs upon differentiation *in vitro* (28). These results suggest the possibility of upregulation of MHC expression in therapeutic ESCs when their use for treatment of ongoing chronic inflammatory diseases or other pathological interferon-γ and similar cytokine abundant conditions. In addition, our recent report supports the argument that interferon-γ may accelerate processing of T cell-reactive self-tumor antigen epitopes presumably *via* upregulation of immunoproteasomes (44), which further emphasizes that stimulations by cytokines, stress, drugs and other stimuli (45) may upregulate the immunogenicity of stem cells by promoting self-antigen epitope processing. Dr. Wu's laboratory tested allogeneic undifferentiated mouse ESCs for their ability to trigger allogeneic immune response in a mouse model of myocardial infarction and observed progressive intra-graft infiltration of inflammatory cells mediating both adaptive and innate immune responses (6). These results suggest that the immunogenicity of mouse ESCs is increased upon their differentiation (6). Moreover, Drs. Mullally and Ritz pointed out that a formerly unappreciated level of "structural variation" within the normal human genome including deletions, duplications, inversions, copy number variants, and single nucleotide polymorphism all increase immunogenicity of transplanted stem cells and affect allogeneic stem cell transplantation (46). Therefore, allogeneic stem cells may not have reliable immune privilege.

In order to generate sufficient numbers of stem cells for therapeutics, isolated stem cells are often required to expand and induced to differentiate *in vitro* (47). For example, to direct autologous adult stem cells into the cardiomyogenic lineage, several strategies have been developed (48) in addition to identification of growth factors and signaling molecules under cell culture conditions (4). Enucleated cytoplasts generated from human ESC-derived cardiomyocytes could be fused with autologous adult stem cells to generate cytoplasmic hybrids or cybrids. Adult stem cells could also be temporarily permeabilized and exposed to cytoplasmic extracts from these cardiomyocytes. Alternatively, intact cells or enucleated cytoplasts from human ESC-derived cardiomyocytes could be co-cultured with adult stem cells *in vitro* to provide the cellular contacts and electronic coupling that might enable some degree of trans-differentiation to take place (48). Long-term *in vitro* culture and manipulations of ESCs (47) may adversely affect their epigenetic integrity including imprinting. Disruption or inappropriate expression of imprinted genes is associated with several clinically significant syndromes and

tumorigenesis in humans. By investigating methylation profiles of CpG sites within the IGF2/H19 IC, Dr. Mitalipov demonstrated abnormal hypermethylation within the IGF2/H19 IC in all analyzed ES cell lines consistent with biallelic expression of these genes (49). Alteration of gene expression leads to changes in antigenic repertoire associated with long-term expansion of autologous stem cells, which may trigger the endogenous "danger signal" sensed by toll-like receptors (50) and activate the host immune system (51). Since the processing of HLA class I-restricted antigen epitope utilizes the ubiquitination-proteasome protein degradation pathway (52, 53) and non-proteasome pathway (54, 55), then, intracellular antigens cannot escape from presenting their epitopes to the HLA class I pathway (56). Theoretically, every antigen with epitope structures blanked by proper processing sites (44) encodes T cell antigen epitope despite the potential variations in the HLA presenting alleles and the differences in their immunodominance [HLA binding affinity (57) and TAP binding affinity (58, 59)] among antigen epitopes (56). Therefore, cellular over-proliferation, tumor formation, and antigenic alteration resulting from *in vitro* expansion of autologous stem cells are potential problems that must be addressed before clinical trials of ESC-based therapy are initiated.

In addition to above-mentioned immune recognition machinery including the expression of MHC molecules, cytokine function, antigen epitope processing, an important question of how nonmutated self-protein antigens, derived from normal stem cells, other normal cells and tumor cells, gain immunogenicity and trigger immune recognition remain poorly defined (56). Mutation may be responsible for elevated immunogenicity underlying some tumor-specific antigens generated *via* mutations (p53 and Ras), chromosome translocations and abnormalities, such as expression of fusion oncogene Bcr-Abl in chronic myelogenous leukemia (CML) (60-63). However, the mechanism underlying the immunogenicity of most non-mutated self-tumor antigens is their aberrant overexpression in tumors. Dr. Zinkernagel et al. (64) suggested that the overexpression of self-antigens or novel antigenic structure, overcomes the threshold of antigen concentration at which an immune response is initiated (65). This threshold might be lower for certain untolerized regions of certain antigen epitopes. Overexpressed genes, up to 100 folds, often encode tumor antigens identified by serological identification of self-antigens by screening expression cDNA library with patients' sera (SEREX) (66), which may reflect the inherent methodological bias for the detection of abundant transcript (67). The overexpression of tumor antigens in tumors can result from transcriptional and post-transcriptional mechanisms. We recently demonstrated that overexpression of tumor antigen CML66L in leukemia cells and tumor cells *via* alternative splicing is the mechanism for its immunogenicity in patients with tumors (68), which not only illustrated the overexpression of tumor antigen as a principle but also elucidated its molecular mechanism (68). In addition, expression of one of the major tumor antigen categories, cancer-testis antigens, in tumors has been ascribed to abnormal demethylation (69, 70).

A significant proportion of the SEREX-defined self-tumor antigens are autoantigens (71), for example, CML28 that we identified is also an autoantigen Rrp46p (72). Beside the overexpression of self-tumor antigens and autoantigens, we also examined the potential mechanisms for non-mutated self-proteins to gain new untolerized structure to trigger immune recognition. We found that alternative splicing occurs in 100% of the autoantigen transcripts. This is significantly higher than the approximately 42% rate of alternative splicing observed in the 9,554 randomly selected human gene transcripts. Within the isoform-specific regions of the autoantigens, 92% and 88% encoded MHC class I and class II-restricted T-cell antigen epitopes, respectively, and 70% encoded antibody binding domains. Furthermore, 80% of the autoantigen transcripts undergo noncanonical alternative splicing, which is also significantly higher than the less than 1% rate in randomly selected gene transcripts. These studies suggest that non-canonical alternative splicing may be an important mechanism for the generation of untolerized epitopes that may lead to autoimmunity. Furthermore, the product of a transcript that does not undergo alternative splicing is unlikely to be a target antigen in autoimmunity (73). To consolidate this finding, we also examined the effect of proinflammatory cytokine tumor necrosis factor-α (TNF-α) on the prototypic alternative splicing factor ASF/SF2 in the splicing machinery. Our results show that TNF-α down-regulates ASF/SF2 expression in cultured muscle cells. This result correlates with our finding of reduced expression of ASF/SF2 in inflamed muscle cells from patients with autoimmune myositis (74). Based on our data, we recently proposed a new model of stimulation-responsive splicing for the selection of autoantigens and self-tumor antigens (45). Our new model theorizes that the significantly higher rates of alternative splicing of autoantigen and self-tumor antigen transcripts that occur in response to stimuli could induce extra-thymic expression of untolerized antigen epitopes for elicitation of autoimmune and anti-tumor responses. Of note, our model is not only applied to non-mutated self-tumor antigens associated tumors and autoantigens associated with various autoimmune diseases, but also applied to composition and expansion of self-antigen repertoire of stem cells. To facilitate the identification of immunogenic isoforms of antigens, we have developed strategies (72, 75-79) using improved SEREX (66) in conjunction with database-mining (73) and immunogenic isoform mapping (68).

However, despite some progress, the detailed definition of antigen repertoire of normal stem cells has not been reported yet. Identification of immunogenic isoforms of autoantigens and self-tumor antigens related to stem cell therapy is very important for the development of novel therapeutics for cell replacement therapy using stem cells (45).

Strategies to inhibit immune rejection to allograft stem cells

Various strategies have been developed to circumvent the

immunological barriers and inhibit the rejection of replacement stem cells. Lessons learned from bone marrow transplantation suggest that it is a formidable task to establish a stem cell bank to permit rudimentary matching of tissue (1). Tissues from $MHC^{-/-}$ mice are rejected by recipients at a rate comparable to their wild-type counterparts (80), suggesting that development of a universal ESC line without expression of its own MHC may not necessarily be beneficial. In addition, transplantation of tissues without expression of MHCs as an immunological surveillance mechanism may create a safe haven for viral infection and malignant transformation of cells (28). Several approaches have been extensively studied to improve the acceptance of transplanted stem cells. Firstly, use of donated oocytes for somatic nuclear transfer (SNT) to create nuclear transfer ESC lines (ntES cells) (81, 82), which are genetically identical to the recipient in all but their mitochondrial genome remaining the preserve of the oocytes themselves (83). The question remains whether mitochondrial proteins might act as a source of minor histocompatibility antigens (84, 85) for transplantation rejection antigens in this case although sharing nuclear genes ensure identity of the MHC haplotype. Secondly, *in vitro* differentiation of ESCs into desirable cell types for the therapy of diseases followed by purification of cardiomyocytes (25) and neurons (86) has been used to achieve better acceptance of allograft. Thirdly, ESC-derived dendritic cells (esDCs, a professional antigen presenting cell type) are implicated in tolerance induction, which share with therapeutic graft the full repertoire of transplantation antigens, and generation of immature esDCs may polarize responding T cells towards a regulatory phenotype (1). Dr. Harrison's group demonstrated a proof of principle that because T cell tolerance can be induced by presenting antigen on resting antigen-presenting cells (APCs), hematopoietic stem cells engineered to express autoantigen in resting APCs could be used to prevent autoimmune disease (87). Proinsulin is a major autoantigen associated with pancreatic β cell destruction in humans with type 1 diabetes (T1D) and in autoimmune non-obese-diabetic (NOD) mice. Syngeneic transplantation of hematopoietic stem cells encoding proinsulin transgenically targeted to APCs totally prevents the development of spontaneous autoimmune diabetes in NOD mice. This antigen-specific immunotherapeutic strategy could be applied to prevent T1D and other autoimmune diseases in humans. Fourthly, tolerance is induced by establishment of hematopoietic chimerism (6). Finally, naturally occurring $CD4^{+}CD25^{high}Foxp3^{+}$ regulatory T cells (Tregs) are differentiated T lymphocytes actively involved in the control and suppression of peripheral immunity (88). Over the past few years, a number of animal studies have demonstrated the critical role of these regulatory T cells in the outcome of allogeneic hematopoietic stem cell transplantation (HSCT). In these models, Tregs can exert a potent suppressive effect on immune effector cells reactive to host antigens and prevent graft-versus-host disease (GVHD) while preserving the graft-versus-leukemia (GVL) effect (89). Building on the results from recent studies, a number of therapeutic strategies are being developed to positively modulate Treg pools *in vivo* and prevent or even correct GVHD. Conversely, clinical interventions can also be envisaged to decrease Treg activity *in vivo* and enhance the GVL effect (89). Along this line, our recent data showed that Tregs regulate T cell responses to self-antigen, and that depletion of Tregs *via* a pro-apoptotic protein Bax-dependent mechanism enhances antigen-specific polyclonal T cell responses. These findings provide support for the idea that stem cell therapy can be improved by therapeutic modulation of survival of Treg cells (90).

Minor histocompatibility antigens (mHA) are allogeneic targets of T cell-mediated graft-versus-tumor (GVT) effects following allogeneic (allo-) stem cell transplantation. Recent research has identified several mHAs as tumor proteins and has also disclosed their unique properties in both the induction and effector phase of GVT reactions. Targeting tumor-specific mHAs by adoptive immunotherapy will prevent tumor tolerance and evoke allo-immune responses, thereby enhancing GVT effects against leukemia and solid tumors (91). Recently acquired knowledge of the role of donor immunization status, new techniques in the generation of mHA-specific cytotoxic T lymphocytes *in vitro*, and innovative principles in vaccination will help to design strategies that exploit mHAs in the immunotherapy of cancer. However, the issue of how to control mHA-mediated immune responses and enhance stem cell allograft requires more work.

Of note, pregnant women have been found to tolerate the unborn conceptus expressing a full set of nonmaternal antigens inherited from the father. The exact mechanisms of immune privilege exhibited by fetal tissues remain poorly defined, which may provide useful insights for future tolerance strategies to improve stem cell allograft acceptance (34).

Immune responses to cancer stem cells

The observation of similarities between the self-renewal mechanisms of stem cells and cancer cells has led to the new concept of the cancer stem cell. In 1994, the presence of cancerous stem cells in acute lymphocytic leukemia was documented by cloning such cells and documenting their self-renewing capacity (92). A self-renewing cancer stem cell population has been identified in solid tumors such as breast (93) and brain (94). These cancer stem cells represent approximately 1% of the tumor and are the only cells in the tumor generating tumors into nude mice (95). In cases of multiple myeloma, cells with a high self renewal potential have also been identified (96). Many researchers now suspect that all cancers are composed of a mixture of stem cells and proliferative cells with a limited lifespan (95). The implications of this research are far reaching. The relapse of many cancers following therapy could be the result of the survival of the cancer stem cells. Therefore, it is critical to fully characterize the immunological features of these cells and to develop immunotherapeutic approaches to eliminate these cancer stem cells without excessive toxicity to normal stem cells. It has been reported that PTEN dependence distinguishes hematopoietic stem cells from leukemia-

initiating cells (97). In this aspect, molecular characterization of cancer stem cells in comparison to normal stem cells suggests a good start. Since intracellular antigens cannot escape from presenting their epitopes to the HLA class I pathway (56), any differences in proteomic composition between cancer stem cells and normal stem cells can be "translated" into antigenic differences.

Tumor immunosurveillance theory suggests that tumors can be recognized and eliminated as a result of natural anti-tumor immune responses that develop in the host (98-101). This argument is supported by the discoveries that: a) the immune system can protect the host against the development of spontaneous and chemically induced tumors; b) the immunogenicity of a tumor is imprinted on the tumor by the immunological environment; and c) individuals with tumor sometimes develop spontaneous reactivity against the antigens of the tumor (98-101). Many influences either from tumor or environment render a tumor either invisible to the host immune system or resistant to the anti-tumor immune responses. Several situations can lead to this result: a) the tumor is non-immunogenic, either because it never expressed any tumor antigens or lost them during tumor development, or the tumor acquired defects in the capacity to present tumor antigens to immune cells; b) the immune system may not be able to recognize or eliminate a tumor because the tumor produces immunosuppressive moieties and induces immuno-suppressive responses (98-102).

A recent review explores similarities between lymphocytes and cancer cells, and proposes a new model for the genesis of human cancer. This model suggests that the development of cancer requires infection(s) in which determinants from pathogens can mimic self-antigens and co-present to the immune system, leading to breaking T cell tolerance. However, autoreactive T cells must be eliminated by apoptosis when the immune response is terminated. Some autoreactive T cells suffer genomic damage in this process, but manage to survive. The resulting cancer stem cell still retains some functions of an inflammatory T cell, so it seeks out sites of inflammation inside the body. Due to its defective constitutive production of inflammatory cytokines and other growth factors, a stroma is built at the site of inflammation similar to the temporary stroma built during wound healing. The cancer cells grow inside this stroma, forming a tumor that provides their vascular supply and protects them from cellular immune response. As cancer stem cells have plasticity comparable to normal stem cells, interactions with surrounding normal tissues cause them to give rise to all the various types of cancers, resembling differentiated tissue types. Metastases form at an advanced stage of the disease, with the proliferation of sites of inflammation inside the body following a similar mechanism. Therefore, future development of cancer therapies should provide more support for, rather than antagonizing, the immune system (103).

Substantial antigenic differences have been found between tumors and normal tissues. A milestone in tumor immunology was the cloning of tumor antigen MAGE-1 by Dr. Boon's team in 1991 (104-106), and subsequent characterization of the first HLA-restricted T cell defined antigenic epitope a year later (107). In 1995, another breakthrough was reported, Dr. Pfreundschuh's team developed a new method of serological cloning approach called SEREX (66, 67, 108, 109). It allows a systemic and unbiased search for antibody responses against protein antigens expressed by human tumors. More than 2,000 tumor antigens have been identified (67) (also see an excellent database http://www.cancerimmunity.org/statics/databases.htm). These advances have led to a renaissance in tumor immunology and studies on anti-tumor immunotherapy (66, 104) (also see our invited reviews (45, 56)).

In addition, studies on identification of HLA-restricted T cell antigen epitopes of tumor antigens and T cell based immunotherapy to tumors have also made significant progress (110). By 2004, more than 257 HLA class I- and HLA class II-restricted T cell antigen epitopes have been identified (http://www.istitutotumori.mi.it/INT/AreaProfessionale/Human_Tumor/default.asp?LinkAttivo=17B). Since they are derived from various tumors, these T cell antigen epitopes are very useful in diagnosis, prognosis, and immunotherapy in treatment of tumors. Furthermore, clinical studies of several formats of active immunization (recombinant viruses, naked DNA, dendritic cells pulsed with peptide, and peptides) in patients with melanoma showed that after two courses of immunization with the gp100, MART-1, or tyrosinase tumor antigens, up to 1-2% of all circulating CD8 T cell had anti-tumor activity, which is several hundred or thousand folds higher than the frequencies of any given antigen-specific T cells in the normal T cell repertoire.

In identifying tumor antigens associated with cancer stem cells, one needs to bear in mind that there are two major groups of self-tumor antigens. The first group comprises conventional antigens, such as proteins encoded by genes with conventional exon-intron organization and translated in the primary open reading frame (ORF) (111). The conventional tumor-associated antigens include the five groups of tumor antigens above-mentioned (112, 113). Our reports on tumor antigens associated with chronic myelogenous leukemia (a myeloid stem cell-initiated hematologic malignancy), such as CML66 (68, 76, 90), CML28 (72), PV13 (79), PV65 (79), and others (75) belong to the first group. The second group comprises unconventional cryptic peptide antigens, including cryptic antigens encoded in a) the introns of genes (68) (MPD associated antigen MPD5) (114), b) the exon-intron junctional regions, c) the alternative reading frames (tumor antigen TRP-1) (115, 116) as opposed to the primary reading frames in mRNAs (117-119), d) the subdominant open reading frames located in the 5'untranslated region (UTR) or 3'UTR of the primary open reading frame (111, 114), chromosome rearrangement, and aberrant processing (110). Recently, we used the SEREX technique to screen a human testis cDNA library with sera from three polycythemia vera (a stem cell-initiated myeloproliferative disease, MPD) patients who responded to interferon-α (IFN-α) and identified a novel unconventional antigen, MPD5. MPD5 belongs to the group of unconventional cryptic antigens without conventional genomic intron/exon structure. MPD5 antigen elicited IgG antibody responses in a subset of

polycythemia vera (PV) patients, as well as some patients with chronic myelogenous leukemia or prostate cancer, suggesting that they are broadly immunogenic. Upregulated expressions of MPD5 in the granulocytes from PV patients after IFN-α (78) or other therapies, might enhance their abilities in elicitation of immune responses in patients. In addition, we recently identified another unconventional antigen MPD6. MPD6 belongs to the group of cryptic Ags without conventional genomic structure and is encoded by a cryptic open reading frame located in the 3'-untranslated region of myotrophin mRNA. MPD6 elicits IgG antibody responses in a subset of polycythemia vera patients, as well as patients with chronic myelogenous leukemia and prostate cancer, suggesting that it is broadly immunogenic. By using bicistronic reporter constructs, we showed that the translation of MPD6 was mediated by a novel internal ribosome entry site (IRES) upstream of the MPD6 reading frame. Furthermore, the MPD6-IRES-mediated translation, but not myotrophin-MPD6 transcription, was significantly upregulated in response to IFN-α stimulation (77). Our findings provide new insights into the mechanism underlying the regulation of the self-antigen repertoire in eliciting anti-tumor immune responses in patients with myeloid stem cell proliferative diseases, and suggest their potential as the targets of novel immunotherapy. What is the significance of identification of unconventional tumor antigens for future immunotherapy? Since these unconventional antigen peptides are not expressed in normal cells and normal stem cells, and are not tolerated by host immune system, they are considered to be tumor-specific or cancer stem cell-specific. These features indicate that these unconventional antigens may be desirable to be targets for future immunotherapy (111).

Despite significant progress in tumor immunology, several important questions remain to be addressed: Firstly, whether there are any differences between the 1% cancer stem cells and the majority of other cancer cells in immunogenicity and antigenic features. Of note, since cancer stem cells represent approximately 1% of the tumor cells (95), tumor antigens highly expressed in cancer stem cells may not be the tumor antigens highly expressed in tumors. The tumor antigens highly expressed in cancer stem cells may have been missed in routine SEREX screening and T cell epitope cloning procedures since immune responses against tumor antigens highly expressed in cancer stem cells are diluted 100 fold during detection. Secondly, whether any identified tumor antigens are specifically upregulated in cancer stem cells in comparison to the majority of other cancer cells. Current anti-tumor antigen-specific immune therapies focused on tumor antigens highly expressed in tumor cells are not capable in elicitation of effective anti-cancer stem cell immune responses and inhibiting cancer stem cell growth and cancer relapse after initial treatment. Thirdly, whether there are any ever changing patterns of transient kinetics of tumor antigen expression in cancer stem cells and other cancer cells. Due to this complicated situation, majority cancer antigen-specific immunotherapy may not be able to be effective alone in eradiating tumors, especially in eradiating cancer stem cells (120). Therefore, future immunotherapy could be in a combinational format, including cancer cell antigen-specific immunotherapy and cancer stem cell antigen-specific immunotherapy as well as anti-tumor immune enhancement therapies including our recently reported promotion of Treg apoptosis (90). In other words, long-term survival of patients with cancer can only be achieved if effective cytotoxic immune responses against both cancer stem cells and cancer cells are established.

Mesenchymal stem cells in immune regulation

Mesenchymal stem cells (MSCs) are adherent, fibroblast-like, pluripotent, non-hematopoietic progenitor cells. MSCs are initially isolated from bone marrow, which constitute 0.001-0.01% of the total cell population (121) and have multilineage differentiation potential (*i.e.*, the ability to differentiate into various tissues of mesenchymal and non-mesenchymal origin) (122-124). MSCs can be easily isolated (125) and found in many different species (122, 123), including humans (126), rodents (127) and primates (128), and in tissues other than bone marrow, including both adult tissues including umbilical cord blood (129), fetal bone marrow, blood, lung, liver and spleen (130), fat (131), hair follicles and scalp subcutaneous tissue (132), and periodontal ligament (133), and pre-natal tissues such as placenta (134). Although there is no agreement on any standardized marker, MSCs are typically defined by a combination of phenotypic and functional characteristics. Using flow cytometry (FCM), human MSCs are negative for hematopoietic markers CD14, CD34 and CD45. Human MSCs are positive in staining for a set of adhesion markers, such as CD44 (135), CD71 (135), CD73, CD90, CD105, and CD166 (21). Similarly, murine MSCs do not express hematopoietic markers CD45, CD34, and CD11b, while they are positive in surface expression of CD9, Sca-1, and CD44 (135). The hallmark of MSCs is the trilineage potential *in vitro* (the ability to differentiate into bone, cartilage and fat upon proper induction) (21). Human MSCs express HLA class I and can be induced by interferon-γ to express HLA class II. However, in co-culture experiments, human MSCs fail to induce proliferation of allogeneic lymphocytes *in vitro*, even after provision of a co-stimulatory signal by addition of CD28-stimulating antibodies or transfection of B7-1 or B7-2 co-stimulatory molecules (21). Several reports showed that MSCs also possess immunoregulatory properties, inasmuch as they can (124): a) Inhibit the function of mature T cells following their activation by non-specific mitogens (136); b) Suppress the response of naïve and memory antigen-specific T cells to their cognate peptide in mice (137); c) Promote the survival of MHC-mismatched skin grafts after infusion in baboons and reduce the incidence of graft-versus-host disease (GVHD) after allogeneic HSC transplantation in humans (138, 139); d) Cure severe acute GVHD refractory to conventional immunosuppressive therapy (140); e) Ameliorate experimental autoimmune encephalomyelitis (EAE) in mice (141). Therefore, we expect that MSCs may join $CD4^+CD25^{high}$ $Foxp3^+$ Tregs in facilitating engraftment of stem cell therapy

for regenerative medicine.

Acknowledgements

I am very grateful to Dr. B. Ashby for critical reading, H. Wang, Y. Yan, Z. Xiong, S. Houser, N. Dun, R. Emmons, and F. London for discussion. This work was supported by grants from NIH and the Leukemia & Lymphoma Society and the Myeloproliferative Disorders Foundation.

References

1. Fairchild PJ, Cartland S, Nolan KF, Waldmann H. Embryonic stem cells and the challenge of transplantation tolerance. Trends Immunol. 2004;25:465-470.
2. Jordan JD, Ming GL, Song H. Adult neurogenesis as a potential therapy for neurodegenerative diseases. Discov Med. 2006;6: 144-147.
3. Gangaram-Panday ST, Faas MM, de Vos P. Towards stem-cell therapy in the endocrine pancreas. Trends Mol Med. 2007;13: 164-173.
4. Sachinidis A, Fleischmann BK, Kolossov E, Wartenberg M, Sauer H, Hescheler J. Cardiac specific differentiation of mouse embryonic stem cells. Cardiovasc Res. 2003;58:278-291.
5. Priddle H, Jones DR, Burridge PW, Patient R. Hematopoiesis from human embryonic stem cells: overcoming the immune barrier in stem cell therapies. Stem Cells. 2006;24:815-824.
6. van der Bogt KE, Swijnenburg RJ, Cao F, Wu JC. Molecular imaging of human embryonic stem cells: keeping an eye on differentiation, tumorigenicity and immunogenicity. Cell Cycle. 2006;5:2748-2752.
7. Faulkner L. Disease Management: The New Tool for Cost Containment and Quality Care: Health Policy Division. NGA Center for Best Practice;2003.
8. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292: 154-156.
9. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A. 1981;78:7634-7638.
10. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145-1147.
11. Young HE, Black AC, Jr. Adult stem cells. Anat Rec A Discov Mol Cell Evol Biol. 2004;276:75-102.
12. Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. Exp Cell Res. 1965;37:614-636.
13. Kiel MJ, Morrison SJ. Maintaining hematopoietic stem cells in the vascular niche. Immunity. 2006;25:862-864.
14. Moraleda JM, Blanquer M, Bleda P, et al. Adult stem cell therapy: dream or reality? Transpl Immunol. 2006;17:74-77.
15. De Coppi P, Bartsch G, Jr., Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol. 2007;25:100-106.
16. Shizuru JA, Negrin RS, Weissman IL. Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. Annu Rev Med. 2005;56:509-538.
17. Cerny J, Quesenberry PJ. Chromatin remodeling and stem cell theory of relativity. J Cell Physiol. 2004;201:1-16.
18. Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. Blood. 2003;102:3483-3493.
19. Goodell MA, Rosenzweig M, Kim H, et al. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nat Med. 1997;3:1337-1345.
20. Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells. 2006;24:3-12.
21. Le Blanc K, Ringden O. Mesenchymal stem cells: properties and role in clinical bone marrow transplantation. Curr Opin Immunol. 2006;18:586-591.
22. Yang ZJ, Wechsler-Reya RJ. Hit 'em where they live: targeting the cancer stem cell niche. Cancer Cell. 2007;11:3-5.
23. Li F, Tiede B, Massague J, Kang Y. Beyond tumorigenesis: cancer stem cells in metastasis. Cell Res. 2007;17:3-14.
24. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes. 2000;49:157-162.
25. Klug MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embronic stem cells form stable intracardiac grafts. J Clin Invest. 1996;98:216-224.
26. Min JY, Yang Y, Converso KL, et al. Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. J Appl Physiol. 2002;92:288-296.
27. Kim JH, Auerbach JM, Rodriguez-Gomez JA, et al. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature. 2002;418:50-56.
28. Bradley JA, Bolton EM, Pedersen RA. Stem cell medicine encounters the immune system. Nat Rev Immunol. 2002; 2:859-871.
29. Nussbaum J, Minami E, Laflamme MA, et al. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. FASEB J. 2007; 21:1345-1357.
30. Swijnenburg RJ, Tanaka M, Vogel H, et al. Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium. Circulation. 2005;112: I166-1172.
31. Modo M, Rezaie P, Heuschling P, Patel S, Male DK, Hodges H. Transplantation of neural stem cells in a rat model of stroke: assessment of short-term graft survival and acute host immunological response. Brain Res. 2002;958:70-82.
32. Zheng XS, Yang XF, Liu WG, Pan DS, Hu WW, Li G. Transplantation of neural stem cells into the traumatized brain induces lymphocyte infiltration. Brain Inj. 2007;21:275-278.
33. Kofidis T, deBruin JL, Tanaka M, et al. They are not stealthy in the heart: embryonic stem cells trigger cell infiltration, humoral and T-lymphocyte-based host immune response. Eur J Cardiothorac Surg. 2005;28:461-466.
34. Fandrich F, Dresske B, Bader M, Schulze M. Embryonic stem cells share immune-privileged features relevant for tolerance induction. J Mol Med. 2002;80:343-350.
35. Li L, Baroja ML, Majumdar A, et al. Human embryonic stem cells possess immune-privileged properties. Stem Cells. 2004;22:448-456.
36. Burt RK, Verda L, Kim DA, Oyama Y, Luo K, Link C. Embryonic stem cells as an alternate marrow donor source: engraftment without graft-versus-host disease. J Exp Med. 2004;199:895-904.
37. Streilein JW. Unraveling immune privilege. Science. 1995;270:1158-1159.
38. O'Connell J, Bennett MW, O'Sullivan GC, Collins JK, Shanahan F. The Fas counterattack: cancer as a site of immune privilege. Immunol Today. 1999;20:46-52.
39. Kennea NL, Stratou C, Naparus A, Fisk NM, Mehmet H. Functional intrinsic and extrinsic apoptotic pathways in human

fetal mesenchymal stem cells. Cell Death Differ. 2005;12:1439-1441.
40. Drukker M, Benvenisty N. The immunogenicity of human embryonic stem-derived cells. Trends Biotechnol. 2004;22:136-141.
41. Drukker M, Katz G, Urbach A, et al. Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci U S A. 2002;99:9864-9869.
42. Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat. 2002;200:249-258.
43. Magliocca JF, Held IK, Odorico JS. Undifferentiated murine embryonic stem cells cannot induce portal tolerance but may possess immune privilege secondary to reduced major histocompatibility complex antigen expression. Stem Cells Dev. 2006;15:707-717.
44. Yang XF, Mirkovic D, Zhang S, et al. Processing sites are different in the generation of HLA-A2.1-restricted, T cell reactive tumor antigen epitopes and viral epitopes. Int J Immunopathol Pharmacol. 2006;19:853-870.
45. Yang F, Chen IH, Xiong Z, Yan Y, Wang H, Yang XF. Model of stimulation-responsive splicing and strategies in identification of immunogenic isoforms of tumor antigens and autoantigens. Clin Immunol. 2006;121:121-133.
46. Mullally A, Ritz J. Beyond HLA: the significance of genomic variation for allogeneic hematopoietic stem cell transplantation. Blood. 2007;109:1355-1362.
47. Oh SK, Kim HS, Park YB, et al. Methods for expansion of human embryonic stem cells. Stem Cells. 2005;23:605-609.
48. Heng BC, Haider HK, Sim EK, Cao T, Tong GQ, Ng SC. Comments about possible use of human embryonic stem cell-derived cardiomyocytes to direct autologous adult stem cells into the cardiomyogenic lineage. Acta Cardiol. 2005;60:7-12.
49. Mitalipov SM. Genomic imprinting in primate embryos and embryonic stem cells. Reprod Fertil Dev. 2006;18:817-821.
50. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. Semin Immunol. 2007;19:3-10.
51. Matzinger P. The danger model: a renewed sense of self. Science. 2002;296:301-305.
52. Rock KL, York IA, Goldberg AL. Post-proteasomal antigen processing for major histocompatibility complex class I presentation. Nat Immunol. 2004;5:670-677.
53. Kloetzel PM. Generation of major histocompatibility complex class I antigens: functional interplay between proteasomes and TPPII. Nat Immunol. 2004;5:661-669.
54. Geier E, Pfeifer G, Wilm M, et al. A giant protease with potential to substitute for some functions of the proteasome. Science. 1999;283:978-981.
55. Luckey CJ, King GM, Marto JA, et al. Proteasomes can either generate or destroy MHC class I epitopes: evidence for nonproteasomal epitope generation in the cytosol. J Immunol. 1998;161:112-121.
56. Yang F, Yang XF. New concepts in tumor antigens: their significance in future immunotherapies for tumors. Cell Mol Immunol. 2005;2:331-341.
57. Altuvia Y, Margalit H. Sequence signals for generation of antigenic peptides by the proteasome: implications for proteasomal cleavage mechanism. J Mol Biol. 2000;295:879-890.
58. Uebel S, Kraas W, Kienle S, Wiesmuller KH, Jung G, Tampe R. Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries. Proc Natl Acad Sci U S A. 1997;94:8976-8981.
59. Daniel S, Brusic V, Caillat-Zucman S, et al. Relationship between peptide selectivities of human transporters associated with antigen processing and HLA class I molecules. J Immunol. 1998;161:617-624.
60. Yotnda P, Firat H, Garcia-Pons F, et al. Cytotoxic T cell response against the chimeric p210 BCR-ABL protein in patients with chronic myelogenous leukemia. J Clin Invest. 1998;101:2290-2296.
61. Pinilla-Ibarz J, Cathcart K, Scheinberg DA. CML vaccines as a paradigm of the specific immunotherapy of cancer. Blood Rev. 2000;14:111-120.
62. Zorn E, Orsini E, Wu CJ, et al. A $CD4^+$ T cell clone selected from a CML patient after donor lymphocyte infusion recognizes BCR-ABL breakpoint peptides but not tumor cells. Transplantation. 2001;71:1131-1137.
63. Clark RE, Dodi IA, Hill SC, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. Blood. 2001;98:2887-2893.
64. Zinkernagel RM. Immunity against solid tumors? Int J Cancer. 2001;93:1-5.
65. Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol. 2001;1:147-153.
66. Sahin U, Tureci O, Schmitt H, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci U S A. 1995;92:11810-11813.
67. Preuss KD, Zwick C, Bormann C, Neumann F, Pfreundschuh M. Analysis of the B-cell repertoire against antigens expressed by human neoplasms. Immunol Rev. 2002;188:43-50.
68. Yan Y, Phan L, Yang F, et al. A novel mechanism of alternative promoter and splicing regulates the epitope generation of tumor antigen CML66-L. J Immunol. 2004;172:651-660.
69. De Smet C, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T. The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. Proc Natl Acad Sci U S A. 1996;93:7149-7153.
70. Gure AO, Wei IJ, Old LJ, Chen YT. The SSX gene family: characterization of 9 complete genes. Int J Cancer. 2002;101:448-453.
71. Chen Y. SEREX review. Cancer Immunity. 2004; http://www.cancerimmunity.org/SEREX/
72. Yang XF, Wu CJ, Chen L, et al. CML28 is a broadly immunogenic antigen, which is overexpressed in tumor cells. Cancer Res. 2002;62:5517-5522.
73. Ng B, Yang F, Huston DP, et al. Increased noncanonical splicing of autoantigen transcripts provides the structural basis for expression of untolerized epitopes. J Allergy Clin Immunol. 2004;114:1463-1470.
74. Xiong Z, Shaibani A, Li YP, et al. Alternative splicing factor ASF/SF2 is down regulated in inflamed muscle. J Clin Pathol. 2006;59:855-861.
75. Wu CJ, Yang XF, McLaughlin S, et al. Detection of a potent humoral response associated with immune-induced remission of chronic myelogenous leukemia. J Clin Invest. 2000;106:705-714.
76. Yang XF, Wu CJ, McLaughlin S, et al. CML66, a broadly immunogenic tumor antigen, elicits a humoral immune response associated with remission of chronic myelogenous leukemia. Proc Natl Acad Sci U S A. 2001;98:7492-7497.
77. Xiong Z, Liu E, Yan Y, et al. An unconventional antigen translated by a novel internal ribosome entry site elicits antitumor humoral immune reactions. J Immunol. 2006;177:

4907-4916.
78. Xiong Z, Liu E, Yan Y, et al. A novel unconventional antigen, MPD5, elicits anti-tumor humoral immune responses in a subset of patients with polycythemia vera. Int J Immunopathol Pharmacol. 2007;20:375-382.
79. Xiong Z, Yan Y, Liu E, et al. Novel tumor antigens elicit anti-tumor humoral immune reactions in a subset of patients with polycythemia vera. Clin Immunol. 2007;122:279-287.
80. Grusby MJ, Auchincloss H, Jr., Lee R, et al. Mice lacking major histocompatibility complex class I and class II molecules. Proc Natl Acad Sci U S A. 1993;90:3913-3917.
81. Munsie MJ, Michalska AE, O'Brien CM, Trounson AO, Pera MF, Mountford PS. Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei. Curr Biol. 2000;10:989-992.
82. Hwang WS, Ryu YJ, Park JH, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. Science. 2004;303:1669-1674.
83. Lanza RP, Chung HY, Yoo JJ, et al. Generation of histocompatible tissues using nuclear transplantation. Nat Biotechnol. 2002;20:689-696.
84. Morse MC, Bleau G, Dabhi VM, et al. The COI mitochondrial gene encodes a minor histocompatibility antigen presented by H2-M3. J Immunol. 1996;156:3301-3307.
85. Simpson E, Roopenian D. Minor histocompatibility antigens. Curr Opin Immunol. 1997;9:655-661.
86. Li M, Pevny L, Lovell-Badge R, Smith A. Generation of purified neural precursors from embryonic stem cells by lineage selection. Curr Biol. 1998;8:971-974.
87. Steptoe RJ, Ritchie JM, Harrison LC. Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes. J Clin Invest. 2003;111:1357-1363.
88. Shevach EM. Certified professionals: $CD4^+CD25^+$ suppressor T cells. J Exp Med. 2001;193:41-46.
89. Zorn E. $CD4^+CD25^+$ regulatory T cells in human hematopoietic cell transplantation. Semin Cancer Biol. 2006;16:150-159.
90. Yan Y, Chen Y, Yang F, et al. HLA-A2.1-restricted T cells react to SEREX-defined tumor antigen CML66L and are suppressed by $CD4^+CD25^+$ regulatory T cells. Int J Immunopathol Pharmacol. 2007;20:75-89.
91. Hambach L, Goulmy E. Immunotherapy of cancer through targeting of minor histocompatibility antigens. Curr Opin Immunol. 2005;17:202-210.
92. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367:645-648.
93. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. Oncogene. 2004;23:7274-7282.
94. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci U S A. 2003;100:15178-15183.
95. Lou H, Dean M. Targeted therapy for cancer stem cells: the patched pathway and ABC transporters. Oncogene. 2007;26: 1357-1360.
96. Tunici P, Irvin D, Liu G, et al. Brain tumor stem cells: new targets for clinical treatments? Neurosurg Focus. 2006;20:E27.
97. Yilmaz OH, Valdez R, Theisen BK, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature. 2006;441:475-482.
98. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21: 137-148.
99. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22:329-360.
100. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991-998.
101. Schreiber RD. Cancer vaccines 2004 opening address: the molecular and cellular basis of cancer immunosurveillance and immunoediting. Cancer Immun. 2005;5 Suppl 1:1.
102. Schreiber H. Tumor Immunology (5th). Philadelphia: Lippincott-Raven Publishers; 2003.
103. Grandics P. The cancer stem cell: evidence for its origin as an injured autoreactive T cell. Mol Cancer. 2006;5:6.
104. van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254:1643-1647.
105. Boon T, van der Bruggen P. Human tumor antigens recognized by T lymphocytes. J Exp Med. 1996;183:725-729.
106. Boon T, Van den Eynde BJ. Cancer vaccines: Cancer antigens. Shared tumor-specific antigens. In: Rosenberg S, ed. Principles and practice of the biologic therapy of cancer (3rd). Philadelphia, Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo: Lippincott Williams and Wilkins; 2000:493-504.
107. Traversari C, van der Bruggen P, Luescher IF, et al. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J Exp Med. 1992;176:1453-1457.
108. Sahin U, Tureci O, Pfreundschuh M. Serological identification of human tumor antigens. Curr Opin Immunol. 1997;9:709-716.
109. Tureci O, Sahin U, Pfreundschuh M. Serological analysis of human tumor antigens: molecular definition and implications. Mol Med Today. 1997;3:342-349.
110. Rosenberg SA. Development of effective immunotherapy for the treatment of patients with cancer. J Am Coll Surg. 2004; 198:685-696.
111. Shastri N, Schwab S, Serwold T. Producing nature's gene-chips: the generation of peptides for display by MHC class I molecules. Annu Rev Immunol. 2002;20:463-493.
112. Scanlan MJ, Jager D. Challenges to the development of antigen-specific breast cancer vaccines. Breast Cancer Res. 2001;3:95-98.
113. Scanlan M, Simpson AJG, Old LJ. The cancer/testis genes: Review, standardization, and commentary. Cancer Immunity. 2004;4:1.
114. Xiong Z, Liu E, Yan Y, et al. A novel molecular mechanism for enhancement of anti-myeloproliferation (MPD) immune responses by interferon-alpha-Identification of two novel SEREX antigens associated with MPD. Blood 2003;102:920a,.
115. Wang RF, Rosenberg SA. Human tumor antigens recognized by T lymphocytes: implications for cancer therapy. J Leukoc Biol. 1996;60:296-309.
116. Schirmbeck R, Riedl P, Fissolo N, Lemonnier FA, Bertoletti A, Reimann J. Translation from cryptic reading frames of DNA vaccines generates an extended repertoire of immunogenic, MHC class I-restricted epitopes. J Immunol. 2005;174:4647-4656.
117. Wang RF, Johnston SL, Zeng G, Topalian SL, Schwartzentruber DJ, Rosenberg SA. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. J Immunol. 1998;161:3598-3606.
118. Mandic M, Almunia C, Vicel S, et al. The alternative open reading frame of LAGE-1 gives rise to multiple promiscuous HLA-DR-restricted epitopes recognized by T-helper 1-type tumor-reactive $CD4^+$ T cells. Cancer Res. 2003;63:6506-6515.
119. Slager EH, Borghi M, van der Minne CE, et al. $CD4^+$ Th2 cell recognition of HLA-DR-restricted epitopes derived from

CAMEL: a tumor antigen translated in an alternative open reading frame. J Immunol. 2003;170:1490-1497.
120. Copland M, Fraser AR, Harrison SJ, Holyoake TL. Targeting the silent minority: emerging immunotherapeutic strategies for eradication of malignant stem cells in chronic myeloid leukaemia. Cancer Immunol Immunother. 2005;54:297-306.
121. Chen X, Armstrong MA, Li G. Mesenchymal stem cells in immunoregulation. Immunol Cell Biol. 2006;84:413-421.
122. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143-147.
123. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418:41-49.
124. Krampera M, Pasini A, Pizzolo G, Cosmi L, Romagnani S, Annunziato F. Regenerative and immunomodulatory potential of mesenchymal stem cells. Curr Opin Pharmacol. 2006;6:435-441.
125. Beyer Nardi N, da Silva Meirelles L. Mesenchymal stem cells: isolation, *in vitro* expansion and characterization. Handb Exp Pharmacol. 2006;174:249-282.
126. Tremain N, Korkko J, Ibberson D, Kopen GC, DiGirolamo C, Phinney DG. MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. Stem Cells. 2001;19:408-418.
127. Phinney DG, Kopen G, Isaacson RL, Prockop DJ. Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. J Cell Biochem. 1999;72:570-585.
128. Devine SM, Bartholomew AM, Mahmud N, et al. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. Exp Hematol. 2001;29:244-255.
129. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol. 2000;109:235-242.
130. in 't Anker PS, Noort WA, Scherjon SA, et al. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. Haematologica. 2003;88:845-852.
131. Lee RH, Kim B, Choi I, et al. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem. 2004;14:311-324.
132. Shih DT, Lee DC, Chen SC, et al. Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. Stem Cells. 2005;23:1012-1020.
133. Trubiani O, Di Primio R, Traini T, et al. Morphological and cytofluorimetric analysis of adult mesenchymal stem cells expanded *ex vivo* from periodontal ligament. Int J Immunopathol Pharmacol. 2005;18:213-221.
134. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells. 2004;22:1338-1345.
135. Uccelli A, Moretta L, Pistoia V. Immunoregulatory function of mesenchymal stem cells. Eur J Immunol. 2006;36:2566-2573.
136. Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002;99:3838-3843.
137. Krampera M, Glennie S, Dyson J, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood. 2003;101:3722-3729.
138. Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. Exp Hematol. 2002;30:42-48.
139. Lazarus HM, Koc ON, Devine SM, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant. 2005;11:389-398.
140. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;363:1439-1441.
141. Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood. 2005;106:1755-1761.