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SCID MOUSE MODELS TO STUDY HUMAN CANCER PATHOGENESIS AND APPROACHES TO THERAPY: POTENTIAL, LIMITATIONS, AND FUTURE DIRECTIONS

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1. ABSTRACT

The successful engraftment of human tumors and human immunocompetent cells into severe combined immunodeficient (SCID) mice has led to the generation of a wide array of different experimental designs that have proven useful in studying the cell biology of human cancer, and for evaluating novel therapeutic approaches to the treatment of cancer. In this review five of the most frequently used embodiments of the SCID model are presented. The goals of this review are to discuss how each model has been utilized to study human cancer and its response to many different novel therapies, to provide an assessment of the strengths and limitations of each model, and to outline future directions with a focus on what is needed to overcome some of the current limitations and pitfalls of the SCID models.

2. INTRODUCTION

The past two decades have witnessed a significant increase in our understanding of the molecular mechanisms of the immune response and the pathogenesis of human cancer. With this knowledge, the approach to cancer therapy has rapidly shifted from an empirical process of anti-cancer drug discovery, to more tumor-specific and less toxic strategies which include protocols based upon immunotherapeutic use of effector cells, antibodies, cytokines, anti-angiogenic drugs, low molecular weight receptor antagonists, enzyme inhibitors and anti-sense oligonucleotides (1). The safety and efficacy of any anti-cancer strategy must ultimately be determined by clinical trials in patients. However, with the rapid increase in the number of new therapeutic approaches to cancer, the development of reliable animal models to screen and preclinically test the potential of these strategies is of critical importance.

Animal models have been developed in which human tumors and human immunocompetent cells are adoptively transferred into immunodeficient mice. Since the first report indicating that severe combined

immunodeficient (SCID) mice could be successfully engrafted with human tumors (2), numerous reports have established that a wide variety of solid human tumors and hematologic neoplasms, both as cell lines and as fresh biopsy tissues, can be engrafted into SCID mice (3, 4). Five years after the first successful engraftment of human tumors, human peripheral blood leukocytes (HuPBL) were shown to engraft following their inoculation into the peritoneal cavity of SCID mice (5). These early observations led to the development of human-SCID mouse chimeric models that have been successfully exploited to evaluate a wide variety of anti-cancer therapies. A brief review of human-SCID mouse models and their use for evaluating immunotherapeutic protocols for human cancer has been reported by us previously (6). In the current article, a more comprehensive review of the SCID models is presented and the limitations and possible solutions to the limitations are considered here. Specific applications of different SCID mouse models to evaluate non-immune-based as well as immune-based strategies for the treatment of human cancer are presented and future applications of SCID models are discussed. To fully appreciate the potential of these models and to better understand their limitations, it is important to begin with a brief review of the biology and genetics of the SCID mouse.

3. RESULTS

3.1. The biology and genetics of the SCID mouse and its response to xenotransplantation

The SCID mouse was derived from mice with a point mutation in chromosome 16 in the CB-17 inbred mouse strain (7, 8). This mutation prevents the accurate functioning of nuclear recombinases, thereby preventing the successful rearrangement of immunoglobulin (Ig) and T-cell receptor (TCR) genes (9, 10). This mutation, which reflects part of a more generalized defect in DNA repair (11), results in the interruption of lymphocyte maturation and a deficit in circulating mature functional T and B lymphocytes. The phenotype is “leaky” since successful rearrangement of Ig and TCR genes and subsequent maturation of lymphocytes do occur in a minority of cells (7, 12, 13). While this “leakiness” is not transmissible in the germline, it increases with age and following irradiation of the mice (14-16). In contrast to the absence of a functional cognitive immunity, SCID mice possess a completely intact innate immune system, with normal numbers of monocyte-macrophages, natural killer (NK) cells and granulocytes (17, 18) which have been shown to suppress or completely prevent the engraftment of some human tissues. This host vs. graft (HVG) response contributes to the variability of engraftment that has been observed from lab to lab and represents a potential pitfall for most human-SCID mouse chimeric models. The development of spontaneous murine thymomas (7) shortens the life span of the mice which limits the duration of *in vivo* experiments. The presence of these thymomas may also be confused with engrafted human tumor xenografts, and complicate the interpretation of results obtained from the SCID mouse model.

Immediately following the inoculation of human tissues (both tumors and leukocytes), SCID mice mount a complex, multistage immune response against the human xenograft involving innate immune cells and soluble factors. The intensity of this host vs. graft response may vary considerably from mouse to mouse, and has also been shown to vary with different histological types of tumors used for engraftment (19). Following intraperitoneal (i.p.) engraftment of human cells, there is a dramatic increase in murine leukopoiesis, a massive neutrophil recruitment into the site of the xenograft, and the induction of several murine cytokines including IL-1ß, IL-4, IL-6, IL-10, IL-12, TNF, and interferons-a, ß and ? that are detected as early as one day post engraftment, and persist for up to two weeks after the transplant (19, 20). Murine granulocytes (19, 20), macrophages (21) and NK cells (22-29) have all been shown to contribute to the inhibition of human cell engraftment.

The reactions of SCID mice to human tissue xenografts must be taken into consideration when attempting to design human-SCID mouse chimeric models and to accurately interpret results obtained using any of the chimeric models to be discussed below. Also, the identification of the murine effector cells and cytokines that are responsible for inhibiting the engraftment of human cells has made it possible to significantly improve the success of human leukocyte- and tumor-xenografts by selectively eliminating or blocking the anti-graft activities of both the murine cells and their soluble products (19, 21, 22, 25 and 29). These approaches are also expected to considerably decrease the variability observed in future uses of the human-SCID chimeric models and will be discussed in the following sections.

3.2. General comments on the design and limitations of human SCID mouse chimeric models

There are five human-SCID chimeric models that are most frequently used for the study of human cancer and for preclinical evaluations of cancer therapies. These are discussed here and are summarized in Table 1. The first four models are developed with the use of human tumor cell- lines and human peripheral blood leukocytes or lymphocyte effector cell lines. In contrast, the xenografts in model #5 are generated by engrafting non-disrupted pieces of human tumor biopsy tissue which includes tumor cells, tumor stroma and inflammatory cells.

In model one, human tumor cell-lines are inoculated via a variety of different routes into SCID mice. Model #1, termed the HuTum/SCID model, has been utilized primarily to study cytotoxic drug-delivery systems such as immunospecific targeting of toxins (30), drugs (31) and radiation (32). More recently, this model has been exploited to study the anti-tumor activity of drugs and antibodies that are designed to interrupt the vascular supply to tumors.

A significant limitation of model #1 has been that monitoring tumor growth required subcutaneous (s.c.) engraftment to permit periodic measurements of the tumor dimensions. This limitation can be overcome by transfecting the tumor cell line with a tumor marker gene (e.g. prostate specific antigen), and monitoring tumor

Table 1. Human/SCID Mouse Chimeric Models for Evaluation of Anti-Cancer Therapies

Model	Engrafted Tissue[a]	Applications[b]	Main Advantage Limitations or Pitfalls	References
#1 HuTum/SCID	Human Tumor Cell Lines	Orthotopic/metastases Drug delivery/therapy (Cytotoxic/antiangiogenic drugs mAb-Direct)	Main advantage is its simplicity. It is limited to evaluating non-immunotherapeutic strategies. Variability of tumor engraftment has been a problem that can be overcome by depletion of mouse NK cells, macrophages and granulocytes. DNA repair defect makes mice sensitive to chemotherapeutic drugs.	30-32, 34-37, 48, 73-92, 126, 127
#2 HumTum x HuPBL/SCID SCID/Winn	Human Tumor Cell Lines + Human PBL	Immunotherapy (active and passive) Identify effector cells	The presence of functional human immunocompetent cells makes it possible to evaluate immunotherapeutic strategies. Presence of human lymphocytes can result in graft vs. host disease and limits ability of these cells to respond to exogenous antigens. Logistically challenging with autologous lymphocytes. Development of spontaneous lymphomas is a pitfall.	51, 52, 54-56, 60, 122, 123, 178
#3 HumTum x Effector/SCID	Human Tumor Cell Lines, + CTL, LAK, D.C. Macrophages NK cells	Immunotherapy (passive) Adoptive Cell Therapy Effect of Cells on Tumor Metastasis	Useful for evaluating homing of effector cells and effects on tumor growth and metastasis in combination with other therapies. Eliminates problem of GVHD, and spontaneous lymphomas seen in Model #2. Cannot study induction of immune response.	103-113
#4 HuPBL-→EBV-Lymph/SCID	Human PBL from EBV sero positive donors	Lymphomagenesis Immunotherapy (passive)	Opportunity to study spontaneously developing human tumor with a well-defined antigen, i.e. the V regions of tumor-associated immunoglobulin light and heavy chains. Can also combine autologous lymphocytes to study immune response to the developed tumor.	5, 59, 60, 134, 135
#5 HuTum x Hu Stroma/SCID	Intact Patient Tumor Biopsy Tissue	Tumor/Stroma Interaction Immunotherapy (active and passive) in situ. Assess TIL function and role of microenvironment	Tumor xenografts resulting from the implantation of fresh non-disrupted tumor tissues contain tumor as well as patients' tumor-associated inflammatory cells that remain viable and responsive to cytokines for prolonged periods. Able to study patients' complex innate and cognitive immune cell response to tumors and the effect of immunotherapeutic protocols in a human tumor microenvironment. Number of mice in an experiment limited by the amount of tumor biopsy tissue available.	63, 64, 92, 159-170

[a] Human PBL and effector cells have been engrafted i.p., s.c. or i.v. The tumor cell lines have been inoculated i.p., s.c., i.v. or into the organ from which they originated i.e. orthotopically. Fresh tumor biopsy tissue is surgically implanted as intact pieces s.c. and fetal stroma engrafted s.c. Abbreviations used here include PBL peripheral blood leukocytes, CTL cytotoxic T lymphocytes, LAK lymphokine activated killer, NK natural killer, D.C. dendritic cells and TIL tumor infiltrating leukocytes. [b] These and other applications of each model are presented and discussed in the text of the article.

growth by quantifying the level of the tumor marker in the SCID mouse serum (33). Using this approach, it has been possible to monitor periodically the effects of cytotoxic drug-loaded immunoliposomes upon the growth and metastasis of human lung tumor xenografts that are established orthotopically in the lung of SCID mice (34). Several orthotopic human tumor xenograft models have been established and successfully exploited to evaluate different therapies (35-37).

Another pitfall associated with model #1 has been the variability from tumor to tumor, and in some cases from mouse to mouse, in the frequency of success in establishing tumor xenografts that grow progressively in the SCID host. The elimination of host NK cells (22-24), macrophages (21, 25, 38), or granulocytes (19, 29), with antibodies have all been successful in enhancing tumor growth and reducing variability in growth patterns and responses to therapy. The effectiveness and the duration of cell depletion are antibody, dose-, and schedule-dependent. Successful depletion is also dependent upon the particular antibody used. For example, it has been shown that mice treated with polyclonal antibodies to asialo GM-1 will recover their NK cell population within about 9 days (39). More recently, a monoclonal antibody (TMβ-1) directed against the murine IL-2 receptor β chain has been developed (40) that will deplete SCID mouse NK cells for at least 6 weeks, resulting in enhanced growth and metastasis of human tumors (39) and improved human leukocyte engraftment (41). While some investigators have used one or more of these remedial host cell depletions, many investigators have not utilized any depletions in their SCID models.

The use of genetically modified SCID mice as recipients provides an alternative approach to the problem of host vs. graft. For example, putting non-obese diabetic (NOD) mice on a SCID background results in mice that have multiple defects in their innate as well as cognitive immune function. The resultant NOD-SCID mice have impaired NK activity, lack of complement activity and have abnormal macrophage function (42). The NOD-SCID mice were shown to have enhanced engraftment and maintenance of human tissue xenografts (43).

An enhancement of the engraftment of human tissues in SCID mice can also be achieved by the sub-lethal irradiation (44), or irradiation in combination with anti-asialo GM-1 treatment (45). Several pitfalls have been recognized with irradiation of SCID mice. These mice are highly radiosensitive (due to their DNA repair defect) and typically develop murine thymomas more frequently after irradiation (46). In addition, irradiation may induce murine lymphocyte development and maturation (47) thus increasing the "leakiness" of the SCID phenotype (15, 16). Radiation also alters the response of tumor bearing mice to both chemotherapy and immunotoxin therapy (44). Thus it would seem prudent, where possible, to avoid irradiating SCID mice. Furthermore, results obtained with irradiated mice should not be equated with those using non-irradiated SCID mice.

The level of engraftment of human tissues has been found to vary with SCID mice from different vendors (44), suggesting that differences in husbandry, genetics or other factors (perhaps microflora and microfauna) may have an impact on experimental results obtained using SCID mice. Some of these non-genetic variables may be controlled by the use of antibiotics in the drinking water and the use of enhanced laminar flow animal racks. It is recommended that all SCID mice should be housed under specific pathogen-free conditions. Our laboratory has utilized a closed colony of C.B-17 SCID mice housed under specific pathogen-free conditions that were derived from over 20 generations of brother x sister matings. Xenografts of human tumors, human peripheral blood leukocytes and tumor infiltrating leukocytes have yielded very reproducible results in frequency of engraftment and response to different therapeutic strategies.

Based upon the DNA repair defect, one would expect that SCID mice would be less able to tolerate many cytotoxic drugs. While this has been reported, the increased toxicity to doxorubicin, for example, can be overcome by scheduling multiple injections at lower doses (48) (similar to what has been observed in humans).

Another embodiment of the SCID model is designed to evaluate a variety of different therapeutic approaches to cancer in which it is necessary to include immunocompetent cells for the assessment of the response. In model #2 human peripheral blood leukocytes (HuPBL) (from normal donors or from the patient) are co-engrafted with human tumor cell lines (i.e. HuTum x HuPBL/SCID). While all of the problems and possible solutions discussed above in model #1 apply to model #2, the engraftment of HuPBL results in additional problems that must be recognized and addressed. HuPBL inoculated i.p. recognize and respond vigorously to murine tissue antigens. This xeno-response, mediated by human B- and T-cells, not only causes potentially fatal GVHD, but severely limits the ability of HuPBL to respond to exogenous antigens (32, 49, 50). The problem has been minimized by coinjecting HuPBL with tumors subcutaneously instead of i.p. (where the HuPBL are restricted from migrating and thus encounter fewer xenoantigens) (51). This approach has been termed the SCID/Winn assay based upon the original Winn model designed for evaluating murine effector cells (52). The SCID/Winn assay has been applied successfully to evaluate novel immunotherapeutic protocols for the treatment of human cancer (51, 53, 54).

While model #2 is quite feasible when using HuPBL from normal donors (i.e. allogeneic with respect to the tumor), the use of patient lymphocytes with their own tumor (i.e. autologous SCID/Winn assay), which is the more desired embodiment, is more challenging logistically. Unfortunately, both the allogeneic and autologous SCID/Winn models lack a true human tumor-microenvironment and, therefore, may not always accurately reflect the biology of human tumors or predict the response to therapy that would occur in patients (see Scid Models of Human Tumor Microenvironments presented below).

The subcutaneous implantation of the HuPBL and tumor minimizes the problem of GVHD and the paralysis of the response of HuPBL to exogenous (non-murine) antigens. However, this protocol does not permit the testing of strategies that are designed to induce and expand a cognitive immune response with memory (such as vaccination strategies). Ideally, one would like to engraft HuPBL first, then vaccinate and allow time for an immune response to develop. Subsequently, mice bearing the activated immunized-HuPBL would be challenged with tumor inoculation at a separate site (preferably engrafted orthotopically with the ability to metastasize). The most optimal site for long term engraftment of HuPBL in SCID mice is i.p. (5). It has now been established that the anti-mouse immune response of HuPBL inoculated i.p. can be inhibited by preventing CD40/CD40L co-stimulation (55). Others have also shown in a SCID model that the blockade of the CD40/CD40L co-stimulatory pathway inhibits the reactivity of HuPBL to differences in MHC antigens (56). These findings suggest that the problem of GVHD in model #2 may be reduced or completely eliminated by inhibiting human CD40/CD40L interaction in the presence of mouse antigens.

Engraftment of HuPBL from EBV seropositive donors results in the spontaneous development of human-B cell lymphomas in SCID mice (5). This represents a potential pitfall with nearly all embodiments of the HuTum x HuPBL/SCID model. Most protocols designed to enhance or promote the engraftment of the HuPBL also potentiate the development of EBV-induced lymphomas (57). A better understanding of the mechanisms involved lymphomagenesis may lead to a means to circumvent this problem in the chimeric-human-SCID model. As discussed above the inoculation of HuPBL into NOD-SCID mice results in an improved level and duration of engraftment. It has also been established the NOD-SCID mice are resistant to the development of human EBV+ lymphomas (58).

Model #3 involves the co-engraftment of human tumor cell lines with isolated subsets of human immunocompetent effector cells, or effector cell-lines that have already been generated and/or activated by the tumor (or tumor antigens) *in vitro*. Model #3, termed HuTumxEffector/SCID, eliminates the problems of GVHD and spontaneous lymphomagenesis that complicate model #2. Model #3 has been used extensively to evaluate, *in vivo*, the ability of tumor-specific cytotoxic T cells (CTL) or CTL cell-lines, macrophages, LAK cells or TIL (isolated from tumor biopsy tissues), either alone or in combination with antibodies or cytokines, to migrate to and kill tumor xenografts. This model, however, is limited to evaluating effector cells and cannot be applied to situations where one is attempting to monitor the induction of an anti-tumor immune response. A distinct advantage of this *in vivo* model over *in vitro* studies of effector cell function is that it provides an opportunity to test the ability of adoptively-transferred effector cells to prevent metastatic spread of the tumor. This model has also been utilized to monitor the homing of human effector cells, and their ability to infiltrate into existing tumor xenografts.

A fourth general model stems from the original observation that the engraftment of HuPBL from EBV-seropositive donors into SCID mice resulted in the spontaneous development of B-cell lymphoproliferative disease (5). The B-cell lymphomas that develop in these mice resemble opportunistic lymphomas (57) that frequently occur in immunosuppressed patients (i.e. transplant recipients and AIDS patients). Model #4, or HuPBL $\rightarrow$ EBV Lymph/SCID model, has contributed to our understanding of lymphomagenesis in humans. One example of this was the finding that human T-cells within the inoculum of HuPBL were required for B-cell lymphoma development (59). This led to the hypothesis that T-cell activation resulted in cytokine production that promoted the expansion of EBV + B-cells (60).

A curious problem related to this fourth model is that many EBV+ lymphomas generated *in vitro* and inoculated subcutaneously into SCID mice spontaneously regress. Some have speculated that this regression is mediated by interferon-γ (IFN-γ) and IFN-γ-inducible protein-10 (IP-10) both of which are provoked by IL-18 expression in response to the EBV latent membrane protein-1 (LMP-1) (61). Thus it may be possible to prevent tumor regression and enhance the engraftment of some B-cell lymphomas with the addition of neutralizing antibodies to one or more of these cytokines or chemokine. Directly relevant and in support of this possibility, it has been reported that IP-10 inhibits the engraftment of human non-small cell lung tumors in SCID mice (presumably due to the anti-angiogenic activity of IP-10) and that neutralization of IP-10 enhanced tumor-derived angiogenic activity, and augmented tumor growth in the mice (62).

All of the four models discussed above are limited by the lack of a human tumor microenvironment, and the use of tumor cell-lines instead of fresh tumor biopsy tissues to establish the xenograft. In Model #5, termed HuTum x HuStroma/SCID model, non-disrupted pieces of fresh human tumor biopsy tissues are surgically implanted into SCID mice (Figure 1). The resultant tumor xenografts are maintained within a human tumor microenvironment. Xenografts established from fresh tumor biopsy tissue consist of the human tumor, and human stromal cells including tumor infiltrating leukocytes (TIL) or inflammatory cells, fibroblasts, extracellular matrix (ECM) and both of human and mouse blood vessels (63). It has been established in this model that the human TIL within the s.c. xenografts of fresh biopsy tissue remain functional and responsive to immunopotentiating cytokines (64). Model #5 provides a unique opportunity to study complex interactive responses of the patients' inflammatory cells *in situ* and over a prolonged period of time. A major strength of this model is its ability to correlate a panoply of molecular and cellular events in response to a defined therapy (for example a local and sustained release of a cytokine) with tumor suppression over a period of weeks or even months. A more detailed discussion of Model #5 and of its specific application are provided in the last section of this review.

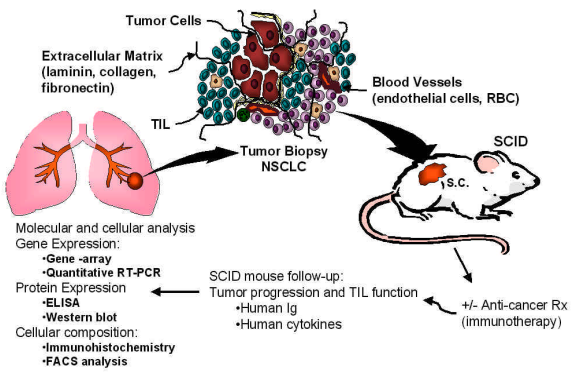

Figure 1. Model 5 for monitoring the effects of anti-cancer therapy on patient tumor xenografts. Following surgical resection of a solid neoplasm, a piece of the fresh tumor biopsy tissue (i.e. pre-engraftment) is saved for molecular and cellular analyses, while the remaining tissue is engrafted as histologically-intact tumor fragments into the subcutis of NK-depleted SCID mice. Each patient's tumor is engrafted into 10-40 mice, establishing xenografts with an intact tumor-microenvironment including both tumor and TIL. One week after engraftment, mice receive anti-cancer treatment (i.e. immunotherapy) or control treatment given either intra-tumorally or systemically. At different time-points post-Rx., the treated-xenografts are biopsied by fine needle aspirate (FNA), or the xenograft is completely removed from the mouse for analysis. Gene expression is analyzed by gene-array and quantitative RT-PCR of tumor-derived RNA; protein expression is assessed by ELISA and Western blots of tumor-lysates; and the cellular composition of the xenografts is determined by immunohistochemistry and FACS. The *in vivo* growth of tumor xenografts is monitored by weekly tumor-measurements, while Hu-TIL function is assessed by quantifying the level of HuIg and human cytokines (e.g. IFN-γ) in SCID mouse sera. The extent of tumor metastasis in SCID mice is determined by necropsy when the experiment is terminated. The patient whose tumor was xenografted is followed clinically for tumor progression and response to therapy. In the event of a tumor recurrence or metastasis, attempts are made to obtain a second biopsy sample for analysis and/or xenografting into additional SCID mice. Correlations among tumor progression in the patient, the cellular and molecular analyses of both the pre-engraftment tumor specimen and tumor xenografts, and the progression in SCID mice are assessed.

During the past decade over 500 papers on the use of the five SCID models for evaluating cancer therapy have been published. Specific examples of the use of the SCID mouse models have been selected and summarized to illustrate the flexibility, utility, and limitations of the models as preclinical tools for testing novel anti-cancer therapies. A conclusion drawn from these summaries is that while none of the SCID models completely recapitulate the biology of the tumor and the immune system in patients, these models do provide valuable insights with respect to therapeutic effects beyond that which is possible with current *in vitro* methods or non-SCID animal tumor models. Moreover, it will also be made evident that improvements can be made in the models to significantly improve upon reproducibility and reliability of data generated from each model.

3.3. Specific applications of human SCID mouse chimeric models to evaluate non-immune-based therapeutic strategies

The SCID mouse models are being used frequently to evaluate novel chemotherapeutic drugs, anti-angiogenic therapies, and several other strategies that are based on non-immunologic approaches. Specific examples of how the SCID mouse has been used to evaluate these therapies are presented and discussed below.

3.3.1. Chemotherapy

SCID mice engrafted with human tumors have been used extensively to evaluate conventional as well as novel chemotherapeutic agents (65-75). Several important variables exist among these studies. For example, several different routes of implanting tumor xenografts have been used. The ectopic route, in which tumor cells are implanted s.c., is the most common. The orthotopic route, where tumors are implanted into the organ in which the tumor originated, is less frequently utilized but may be the more relevant model for studying the drug effects upon tumor progression and metastasis. In some models extracellular matrix has been combined with tumor cells before they are implanted into the host to improve engraftment and metastasis (76, 77).

A number of different established human tumor cell-lines (i.e. lung, colon, lymphoblastoid, leukemia, prostate, melanoma and breast) as well as early passaged breast cancer and melanoma cells have been inoculated ectopically or orthotopically into SCID mice and used to test the ability of conventional chemotherapeutic drugs to suppress tumor growth (31). Drugs that are known to be clinically effective for a particular tumor-type were shown to be efficacious in the HuTum-SCID model. For example, Tamoxifen and cyclophosphamide which are both effective in breast cancer patients suppressed the growth of breast tumor xenografts either established s.c. or orthotopically in the mammary fat pad of SCID mice (31). SCID mouse leukemia models also reflected clinical results by showing significantly reduced mortality in doxorubicin-treated mice bearing leukemic cells. In colon cancer and melanomas that were clinically resistant to chemotherapy the tumors were also unresponsive to chemotherapy in the SCID mouse model. These results suggest that the SCID models correlate with what has been observed in cancer patients and, therefore, may be useful for *in vivo* testing of new chemotherapeutic agents against several different tumor types.

Tumor xenografts in SCID mice have also been used to evaluate novel drug-delivery systems. For example, this model was used to demonstrate the clear superiority of sterically-stabilized liposomes over conventional liposomes for the delivery of doxorubicin to lung tumor xenografts (78, 79).

3.3.2. Anti-angiogenic therapies tested in SCID mouse models

SCID mice bearing human tumor xenografts have been exploited to test various therapeutic

strategies aimed at inhibiting tumor vascularization. For example, a monoclonal antibody to integrin-αvβ3 (expressed on human endothelial cells) blocked angiogenesis and human breast cancer growth following injection of tumor cells into a human skin xenograft (80). This finding is significant since the blood vessels supplying the tumor within the xenografted skin were found to be of human origin (80). Antibodies to endoglin, a molecule expressed on the surface of proliferating endothelial cells, have been used to deliver toxins (ricin A chain) (81) and radionuclides (^{125}I) (82) to the blood vessels supplying human tumor xenografts. However, the vasculature inhibited in these reports was derived from the SCID mouse host (81, 82). In addition to targeting endothelial cells, specific monoclonal antibodies were found to neutalize soluble pro-angiogenic factors including VEGF (83), and the ELR+ chemokines IL-8 (84), ENA-78 (85), and GRO-α (84, 86), resulting in a decrease in angiogenesis, and a decrease in the growth and metastasis of human tumor xenografts. Streiter and colleagues demonstrated a significant inhibition of tumor growth and neo-vascularization following the introduction of anti-angiogenic chemokines, IP-10 (60) and MIG (87), to the microenvironment of human tumor xenografts. Pre-clinical screening of novel anti-angiogenic drugs can be accomplished easily in the Hu-Tum/SCID model. Two such drugs, Linomide (88) and TNP-470 (89, 90), have been shown to inhibit angiogenesis and decrease the growth of human tumor xenografts *in vivo*. Nutritional compounds, and the effects of diet on tumor-angiogenesis, are now being investigated in SCID mice inoculated with human tumor cell-lines. Inhibition of human prostate tumor growth and angiogenesis was observed when mice were fed a soy-protein isolate (91), or a diet reduced in energy intake (old 89 now 92).

The major limitation associated with testing anti-angiogenic therapies in SCID mice include the murine-origin of the vascular endothelial cells (except in cases where human skin or intact tumor tissue is engrafted). In addition, drugs and dietary compounds may be metabolized differently in mice than in humans. Finally, this model fails to account for the possible immune response to foreign antibodies.

3.3.3. Other Non-Immunological Therapies

Introduction of the thymidine kinase or cytosine deamidase genes into tumor cells results in the conversion of systemically administered prodrugs ganciclovir and 5-fluorocytosine into their respective toxic forms which kill both transfected and bystander tumor cells (93). The HuTum/SCID model has provided a useful *in vivo* platform to test the efficacy of this approach in the treatment of hematological malignancies (94) and solid tumors (95, 96). While the model has been useful in predicting the general anti-tumor efficacy of this strategy, it does not address some of the questions associated with viral gene transfer, i.e. the development of antiviral immune responses in patients and toxicity problems associated with the infection of normal human tissues by viral vectors.

Other non-immune mechanism-based therapies that have been tested in the HuTum/SCID model include transfer of apoptosis inducing genes to tumor cells (97), use of protein kinase inhibitors (98), matrix metalloproteinase inhibitors (99), use of synthetic steroids (100), urokinase inhibitors for treatment of prostate cancer (101) and DNA topoisomerase inhibitors (102).

3.4. Specific examples of human SCID mouse chimeric models used to evaluate immune-based cancer therapies

3.4.1. Assessment of effector cells

One of the earliest successful uses of the HuTumxEffectorSCID model (i.e. Model #3) established that human cytotoxic T-cells were able to suppress the growth and spontaneous metastases of human melanomas (103). The suppression of both primary tumor recurrence after surgical resection, and tumor metastases, sustained the ability of the adoptively-transferred CTL to home to and suppress tumor growth. It was further established that exogenous IL-2 enhanced the CTL-mediated anti-tumor immunity (103). Whenever human cytokines are used that are known to be biologically active on murine cells (i.e. IL-2) it is important to establish whether murine cells are contributing to the anti-tumor effect. In this particular case, the authors ruled out the possible contribution of SCID NK and LAK cells (103). Unfortunately, several previous studies have failed to address the role of host effector cells and cytokines in mediating the outcome of selected therapies and therefore conclusions drawn from these studies are left open to question.

The HuTumxEffector/SCID model was also used to test the ability of antibodies to direct the delivery of lymphokine activated killer (LAK) cells to human tumor xenografts (104). Mice with established tumors were treated with either LAK cells alone, or with LAK cells linked with anti-tumor antibodies. LAK cells alone did not inhibit the tumor xenograft, but the addition of antibody resulted in the increased uptake of LAK cells into the tumor xenograft, resulting in decreased tumor growth and increased survival of the mice (104).

A human MHC non-restricted cytotoxic T cell line, TALL-104, was found to display cytotoxic activity against a broad range of tumors while sparing cells from normal tissues. The developers of this cell line used a SCID model to show that irradiated TALL-104 cells administered i.v. into SCID mice, suppressed established xenografts of several different types of human tumors inoculated s.c. or i.v. (105). These investigators attempted to eliminate the effects of SCID NK cells, macrophages and granulocytes in their studies by pretreating their mice with atoposide (105). On the basis of these preclinical studies, a phase 1 trial of TALL-104 cells in patients with refractory metastatic breast cancer was initiated (106).

Additional studies have confirmed that both allogeneic (107-109) and autologous (110) human tumor-specific CTL can suppress the growth of several different tumor types. While all of these papers showed some tumor suppression, differences in the levels of suppression and the requirements for cytokines were noted. Some of these differences are likely due to differences in the protocols used. For example, the diminished functional activity of CTL recovered from SCID mice in one paper could be due to the failure to deplete the innate immune reactive cells in the host (108). In one report GVHD was observed (107)

but not reported in others (108-110). The SCID mouse has been utilized effectively to test some rather novel effector cells. For example, model #3 was used to demonstrate the efficacy of murine T cells that were genetically engineered to kill human tumor cells (111, 112).

3.4.2. Cytokine and chemokine-based immunotherapies

The simplest model that has been utilized for evaluating cytokines and chemokines is the HuTumor/SCID (or Model #1), which takes advantage of the fact that the SCID mice possess functional NK-cells, macrophages and polymorphonuclear cells that can be activated by both human and mouse cytokines to suppress the growth of human tumor xenografts. While this is a more contrived system, it provides a simple initial screen for evaluating cytokine-based strategies targeting innate responses. Cytokines that have been tested in this model include IL-2, IL-10, IL-12, IL-18 and Flt3 ligand which were shown to induce their anti-tumor effects primarily through murine NK cells (113-121). Serious limitations of this model include the xenogeneic nature of the anti-tumor effector cells and the inability to evaluate cognitive immunity.

The HuTum x HuPBL/SCID model (Model #2) provides the opportunity to evaluate human cytokines which augment both innate and cognitive anti-tumor immune responses of human leukocytes (and do not act on murine effector cells), thus providing a model that is more clinically relevant. This model has been utilized to test the anti-tumor efficacy of a wide spectrum of human cytokines including IL-6, IL-12, IL-16 and GM-CSF (54, 122, 123). Strategies involving the delivery of cytokines in solution (56), by gene-transfer (122, 123, 124) and from slow-release polymer particles (biodegradable microspheres) (53, 54, 178) have been evaluated. In the overwhelming majority of these studies, the HuPBL were allogeneic with respect to the tumor and the allo-anti-tumor response was found to be mediated primarily by CD8+ T- and CD56+ NK-cells. In two studies a similar augmentation of an autologous anti-tumor immunity was also demonstrated (53, 178). The literature on the use of chemokines in human/SCID chimeras is largely limited to anti-angiogenic chemokines (discussed above). However, in one study, the anti-tumor potential of monocyte chemoattractant protein-1 (MCP-1) was investigated in the HuTumor/SCID model and was shown to mediate a murine NK-cell- and monocyte/macrophage-dependent suppression of MCP-1 transfected human lung tumor cell metastasis (125).

3.4.3. Antibody-based therapies tested in SCID-hu models of cancer

3.4.3.1. Anti-tumor antibodies

The most basic antibody therapy involves the treatment of SCID mice bearing human tumor xenografts with systemic injections of monoclonal antibodies directed against molecules expressed by the tumor cells. Tumor-cell lines derived from hematopoietic malignancies are often used, as the cell surface molecules expressed by these cells are well characterized (e.g. CD20, CD7, CD40, CD45, CD52, etc.) (126-128). Other molecules targeted by anti-tumor antibodies include CEA (129), P-glycoprotein (130), and the ganglioside, GM2 (131). Monoclonal antibodies used for therapy are usually made in mouse (128) or rat (127), and are injected as intact immunoglobulin molecules. In some cases, chimeric mouse-human antibodies (129-132), consisting of murine variable (V)-regions and human constant (C)-regions, are employed since “humanized” antibodies are thought to be preferable for treating cancer patients clinically. The mechanism responsible for the anti-tumor effect observed in these systems is antibody-dependent cellular cytotoxicity (ADCC), mediated either by resident host cells (e.g. NK, macrophage, eosinophils) (126-128, 130, 131), or by adoptively-transferred human effector cells (PBL,LAK) (129, 132) or a combination of both. Of specific note is the use of anti-human CD40 antibodies for the treatment of human B-cell lymphoma xenografts, since ligation of CD40 on lymphoma cells has direct anti-proliferative effects in addition to mediating ADCC (126, 133). Anti-CD40 treatment has also been shown to inhibit lymphomagenesis in the HuPBL $\rightarrow$ EBV lymphoma/SCID mouse model (134, 135). It is important to emphasize that results obtained from *in vivo* antibody therapy in SCID mice are critically-dependent upon the effector cell(s) involved.

The use of human tumor xenografts in SCID mice to evaluate the effects of antibodies on tumor progression have led to some interesting and unexpected findings that could not have been made with *in vitro* studies. One recent example is the finding that the acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies was due in part to the increased expression levels of vascular endothelial growth factor (VEGF) in tumor variants that progress under the pressure of anti-EGFR antibody therapy (136).

3.4.3.2. Bi-specific antibodies

The use of bi-specific antibodies to target human effector cells to the site of the tumor have also been examined using human-SCID chimeric models (137-140). In this scenario, the monoclonal antibody is engineered to have dual-specificity for a molecule expressed by the tumor (CD19, c-erb-B2, etc.) and a marker on human leukocytes (CD3, CD28, CD16, etc.). As such, the co-administration of antibody and hu-effector and tumor cells to SCID mice result in the targeting of the hu-effector cells to the tumor xenograft, leading to tumor-regression (Models #2 and #3). Similarly, tumor-reactive antibodies fused to a bacterial superantigen (staphylococcal enterotoxin A) induce the recruitment and activation of adoptively-transferred human PBL or LAK cells to human tumor xenografts in SCID mice (141-143). Data from these studies show that human T-cells (CD4+ and CD8+) and macrophages are recruited to the site of the tumor where they produce a variety of human cytokines (e.g. TNF-α, IFN-α, IL-2, IL-12, etc.) resulting in tumor regression (142).

3.4.3.3. Immunocytokines

Antibody-cytokine fusion proteins (immunocytokines) have been tested in SCID mice with established human tumor metastasis. Targeting of either human IL-2 (115, 144) or human IL-12 (145) to the site of the tumor inhibited the growth of metastatic human tumor

cells and extended the survival of xenografted animals. In all of these Model #3 studies, SCID mice were reconstituted with human LAK cells which were required for the anti-tumor effect of IL-12 immunocytokine therapy (145). However, the anti-tumor efficacy of antibody-IL-2 therapy was not dependent on human LAK and was shown to be mediated by murine macrophages (115, 144).

3.4.3.4. Immunotoxins/Immunoliposomes

Numerous immunotoxins targeting leukocyte surface antigens, including CD3, CD7, CD19, CD22, CD25, CD30, CD38, CD40, CD54, and GM-CSF receptor, have been tested in model #1 for the treatment of various human hematological malignancies engrafted in SCID mice (29, 146). Other targets that have been tested include heat shock proteins and endoglin (81, 147). These models have provided justification for evaluating a number of these compounds in the clinic. A novel human skin xenograft/SCID mouse model for testing effects of immunotoxins on vascular leakage has been reported and may be useful for determining the effect of different immunotoxins on vascular leakage in humans (148).

Targeted delivery of chemotherapeutic drugs encapsulated into immunoliposomes is another area where Model #1 has been utilized successfully. The anti-tumor efficacy of drug-containing antibody-conjugated liposomes that target human lymphoma, breast and lung tumor xenografts in SCID mice have been tested. These studies have shown that immunoliposomes represent a superior alternative to free drug or drug-encapsulated non-targeted liposomes for the suppression of established tumors, and perhaps more importantly, the prevention of metastasis (34, 149-151).

A serious limitation to all studies of antibody-based therapy in SCID mouse models of cancer is the lack of normal human tissue to assess antibody cross-reactivity/toxicity. In addition, the SCID mouse host does not generate an immune response against the therapeutic antibody as would be expected in immunocompetent human patients. It is also important when assessing the results of antibody therapies in the SCID mouse models to take into consideration the isotype of the antibody, whether the intact antibody or antibody fragment was used, what murine cells were depleted and which, if any, human cells were added.

3.4.3.5. Co-stimulatory molecule-based immunotherapies

The HuPBL/SCID mouse Model #2 has been effectively utilized to study the role of co-stimulatory molecules CD28, CD80, CD86, CD40 and CD154 in the development of human immune responses against allo- and xeno-antigens (55, 56, 152-154). On the other hand, very few studies have been done in Model #2 to investigate the role of co-stimulatory molecules in tumor immunity. In one study transfection of CD86 into human tumors resulted in enhanced suppression of both CD86-transfected primary human tumor xenografts and distant non-transfected tumors by allogeneic HuPBL in SCID mice (155). In another study, the potential of the co-stimulatory molecule 4-1BB as a target for augmentation of anti-tumor immunity was investigated in Model #2 (156). In contrast to the results obtained in syngeneic murine tumor models, administration of an anti-4-1BB monoclonal antibody abolished the HuPBL-mediated suppression of allogeneic human tumor xenografts.

Targeting of CD40-expressing human B-cell lymphomas with anti-CD40 antibodies inhibits the growth of these tumors in SCID mice (see section on antibody-mediated immunotherapies). In a similar approach, administration of soluble CD40 ligand trimer (CD40Lt) to SCID mice bearing CD40 positive human breast tumors was shown to suppress tumor growth by inducing apoptosis in ligated tumor cells (157). However, this phenomenon does not appear to be generalizable to all human tumors since CD40Lt had no effect upon the growth of CD40+ human lung tumor xenografts in SCID mice (158).

3.5. SCID Mouse Models to Study Human Tumors Within a Human Microenvironment

A major deficiency of the first four SCID models is their lack of a human microenvironment, which is now recognized to play a critical role in regulating tumor growth, metastasis, and in the response to immunotherapy. In view of this, Model #5 has been developed which allows human tumor cells to be engrafted into SCID mice in the context of a human microenvironment. These models systems are discussed below (Figure 1).

3.5.1. Artificial Tumor Microenvironments established by coengrafting tumors with normal human tissues

Engraftment of human cancer cells in the context of a human microenvironment was first designed as a two-step process whereby SCID mice were first engrafted with normal human tissue primarily derived from fetal organs followed by direct inoculation of human cancer cells into the fetal xenograft (159-161). Fresh cells from human T-cell non- Hodgkins lymphoma patients grew within human thymus xenografts, but not in murine thymus tissue, thus demonstrating the species-specificity of this tumor cell/stroma interaction (159). A similar model was developed to study the homing of multiple myeloma cells to the human bone marrow microenvironment (161). After engrafting SCID mice bi-laterally with fragments of fetal bone tissue, human myeloma cell lines were injected into one of the two bone-xenografts. Metastasis to the marrow of the other human bone-xenograft was observed, while no human cells were detected in the marrow of the SCID mouse host (161).

In vivo metastasis of human lung cancer (162) and colon cancer (163) cell lines has also been studied using SCID mice engrafted with a surrogate human microenvironment. After establishing fetal lung or bone marrow xenografts, tumor cells were injected i.v. and found to metastasize in a species-specific and tissue-specific manner. An important observation discussed in this paper is that the cellular composition of the human fetal xenografts in SCID mice changes over time and can influence the engraftment of tumor cells (162).

Other models have been developed in which normal human skin (fetal or adult) is first transplanted into

the backs of SCID mice, followed by direct injection of human cancer cells into the skin xenograft (164). More recently, direct UV-irradiation of human skin xenografts resulted in the induction of human skin tumors *in situ* (165).

These models are ideal for comparing the growth and metastasis of human tumors within a variety of different human microenvironments (e.g. thymus, bone marrow, spleen, liver, lung, skin, etc.). In addition, such models are well suited for studying the mechanisms involved in human tumor cell homing/metastasis, including the role of soluble factors (cytokines) and adhesion molecules in the growth and dissemination of human tumors. The model can also be utilized to test therapies aimed at inhibition of human tumor cell proliferation, metatasis, or angiogenesis in the context of a human tissue microenvironment.

One technical (and ethical) limitation of this model is the need to acquire adequate quantities of human fetal tissue. The introduction of tumor cells, which are allogeneic with respect to the fetal tissue xenograft, results in another potential pitfall, especially in the case of fetal organ xenografts containing significant numbers of leukocytes (i.e. thymus, spleen and bone marrow) or when human tumor cells are of hematopoietic origin.

Fetal tissues often differ significantly from the corresponding adult tissue both phenotypically and functionally. In particular, the presence of immature or undifferentiated stromal cells in fetal tissue is likely to result in a different expression pattern of cell-surface molecules, extracellular-matrix proteins, and soluble factors, than would be found in fully-mature adult tissue. Furthermore, in contrast to the mature well-formed blood vessels which supply normal tissue, the vasculature which supports a solid tumor is characterized by leaky, tortuous, poorly-developed blood vessels which are in a dynamic state of angiogenesis (166). The absence of hu-TIL in the fetal stroma precludes the use of this model for the study of patients' natural immune response to their tumors and the testing of "Active Immunotherapy" protocols.

3.5.2. Natural Human Tumor Microenvironments Established by Engrafting Fresh Non-Disrupted Human Tumor Biopsy Tissue

In contrast to artificial microenvironments, the engraftment of fresh non-disrupted pieces of tumor tissues into SCID mice result in a xenograft in which the tumor is surrounded by an autologous human tumor microenvironment that includes stromal cells, extracellular matrix, a vascular bed including both murine and human blood vessels and most significantly an array of human inflammatory cells or tumor infiltrating leukocytes (TIL) (Figure 1). This approach offers several advantages over the use of fetal-tissue surrogate microenvironments. The tumor and stromal-microenvironment are from the same individual and, thus, histocompatibility differences are not a concern. The elements of a "natural tumor stroma" that are present within the microenvironment, including tumor-associated ECM, vasculature have evolved in place with the tumor and human tumor-infiltrating leukocytes (hu-TIL) that have homed to the site of the tumor are likely to be tumor-specific/tumor-reactive (Figure 1). Because the tissue is surgically implanted into SCID mice with minimal *in vitro* manipulation (other than cutting tissue into fragments), the original local architecture of the tumor specimen is not disturbed and can be maintained in the xenograft for up to 22 weeks (63).

The first engraftment of non-disrupted pieces of human tumor tissue revealed that the subcutaneous implantation of intact human lung tumor specimens into SCID mice resulted in the successful engraftment of both tumor and infiltrating leukocytes (TIL) (32). The histology of the xenografts and the presence of human immunoglobulin (huIg) in the sera of xenografted mice served as an indicator of human TIL engraftment in this study (32). In another early study, Charley *et. al*. transplanted lesional-skin from a patient with cutaneous T-cell lymphoma (CTCL) and monitored the graft for human CD4+ leukemia cells. The human leukemia persisted within the lesional-skin graft for a period of one month after transplantation (168). The fact that the xenografted skin maintained the original histopathologic features observed in the patient suggested that the human tumor microenvironment was preserved following engraftment into SCID mice.

Subsequently, Lubaroff *et al.* demonstrated that the intact surgical specimens of human prostate cancer grew following subcutaneous implantation into SCID mice (169). The growth of these prostate tumor-xenografts was enhanced by the presence of endogenous murine androgens, as well as exogenously administered dihydrotestosterone (169). Importantly, the xenografts displayed the same pathology as the original patient specimens and continued to secrete human PSA. Engraftment of non-disrupted pieces of human breast tumor tissues into SCID mice has been reported (170) and a similar SCID mouse model was used to examine the microenvironment of human papillary thyroid carcinoma (PTH) specimens (171). This later study revealed that human TIL within the tumor-microenvironment secreted anti-thyroglobulin (Tg-Ab) antibodies and augmented the expression of HLA-DR on thyroid epithelial tumor cells (171).

The surgical implantation of non-disrupted human lung tumor tissue (Figure 1) revealed the following: (1) Successful engraftment of hu-TIL occurs in more than 95% of patient tumors xenografted, regardless of the histological type of tumor (63); (2) Human TIL remain at the site of the xenograft and show evidence of proliferation *in situ* (63); (3) The huIg present in the SCID mouse sera is primarily IgG (167) and is produced by human plasma cells in T-cell dependent manner (63); (4) Sera from xenografted mice contain antibodies that react against proteins derived from autologous tumor tissue (63, 167), as well as allogeneic lung tumor cell-lines (167), but not normal lung tissue (167); (5) High levels of huIg in the sera of mice engrafted with tumor biopsy tissue are associated with the growth arrest of adenocarcinoma xenografts (63); and (6) Serial passage of lung tumor

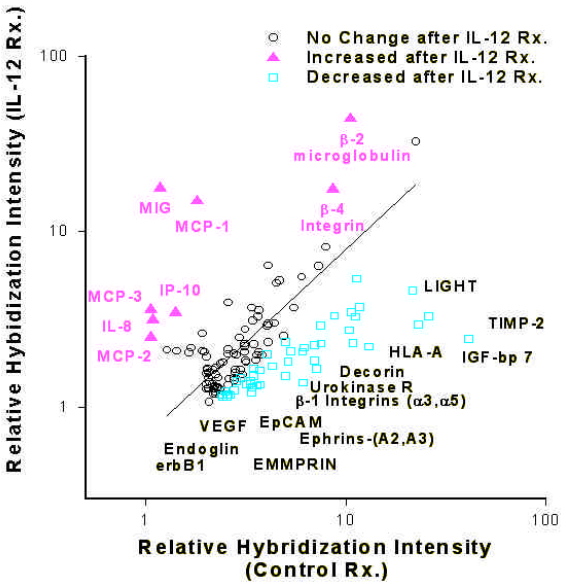

Figure 2. Gene expression patterns in human tumor xenografts after treatment with either control BSA loaded microspheres or BSA plus IL-12 loaded microspheres. Data obtained from gene array analysis of human lung tumor xenografts following treatment *in vivo*. Xenografts were established s.c. in SCID mice from a patient lung tumor biopsy specimen, and treated one week later by direct intra-tumoral injection of biodegradable microspheres loaded with recombinant human IL-12, or control (cytokine-free) BSA-loaded microspheres. Two days after treatment, xenografts were removed for isolation of total RNA. Gene array analysis was performed using a human cytokine expression array from R and D Systems, Minneapolis, MN. The average background value was subtracted from the signal of each spot. Signals were then normalized by correcting for the total signal-intensity of all the spots on the arrays. The normalized, average signal-intensity from each gene expressed in both the control-treated xenograft and the IL-12 treated xenograft was plotted against one another. Symbols that lie above the regression line represent genes more highly expressed in the IL-12-treated xenograft, while symbols below the line indicate genes more highly expressed in the control-treated xenograft. Note that the majority of the >200 expressed genes are not affected by injection of human IL-12 into the xenograft. However, the expression of a number of genes is either increased or decreased as a result of IL-12 treatment.

xenografts in SCID mice results in the loss of hu-TIL from the tumor microenvironment and the disappearance of huIg from the SCID serum (63).

3.5.3. Human Tumor Associated Inflammatory Cells within the Microenvironment of Tumor Xenografts Established from Fresh Biopsy Tissues Remain Viable and Responsive to Cytokines

In xenografts established by the implantation of fresh non-disrupted tumor tissues from human non-small cell lung tumors the patients' tumor cells co-exist with patients' inflammatory cells (including CD4+ T cells, CD8+ T cells, CD19+ B cells with a minority of NK cells, monocytes and macrophages). If left untreated the tumor cells in the primary xenograft continue to grow progressively faster and ultimately outgrow the inflammatory cells. And when these tumors are transplanted these secondary xenografts consistently grow more rapidly in the absence of the inflammatory cells. However, with the local and sustained release of a proinflammatory cytokine (IL-12) from biodegradable microspheres, the inflammatory cells are maintained and expanded and they are able to generate an anti-tumor response that leads to the arrest of the tumor xenograft (64).

The molecular and cellular events that occur within the tumor microenvironment following the introduction of IL-12 have been monitored and correlations of these events with tumor arrest have been established. Using gene expression arrays changes in gene expression patterns in the tumor microenvironment reveal that human IL-12 induces the upregulation of many different proinflammatory cytokines, chemokines, activation markers and their receptors (Figure 2). These changes in gene expression patterns and tumor suppression are causally linked to the production and release of human gamma interferons from CD4+ T cells in the microenvironment. The molecular and cellular events that occur within the tumor following IL-12 release are mediated by the human inflammatory cells and are consistent with both an innate and a cognitive immune response.

The established tumor microenvironment within the xenografts is most likely critical for maintaining the complex events that have been observed in Model #5. It is becoming increasingly apparent that the extracellular matrix and stromal cells within microenvironments play a key role in programming immunocompetent ecells and coordinating T-cell activation and migration of both leukocytes and tumor cells (174-177). In view of these findings, the ability to preserve human microenvironments by implanting fresh non-disrupted tumor biopsy tissue into SCID mice represents a novel and viable opportunity to evaluate cancer immunotherapeutic strategies and to recognize and quantify changes in the microenvironment and correlate these changes with tumor progression. It is expected that these studies will be able to document both direct cell mediated tumor cytotoxic activity but also will be able to recognize more subtle and indirect events that may contribute to tumor arrest. One example of the latter is the finding that IL-12 induced activation of two chemokines IP-10 and MIG. In addition to their ability to attract activated T cells, these molecules have angiostatic activity and may contribute to tumor arrest by disrupting the vacular supply to the tumor.

Model #5 does have its limitations. The amount of biopsy tissue is often limited, thus restricting the number of mice that can be engrafted. This becomes a problem when individual mice must be sacrificed in order to remove the entire xenograft for analysis. This problem may be resolved in part by the use of fine-needle aspirates (FNA) (172) to periodically biopsy the tumor xenografts without removing them from the SCID mouse host. It has been

determined that sufficient quantities of RNA can be obtained from the FNA for both RT-PCR and gene array analyses, thereby providing the opportunity to monitor changes in gene expression without sacrificing mice. Since mice are not sacrificed, the changes in gene expression can be correlated with tumor progression. Variations of this model in which PBL or cells from a tumor draining lymph node from the same patient are injected intravenously into SCID mice bearing established tumor xenografts will make it possible to assess the ability of therapeutic protocols to provoke the recruitment of additional inflammatory cells (TIL) into the tumor microenvironment and to monitor the effects of this recruitment upon tumor growth.

3.6. Future Directions

Multiple mechanisms both immunologically and non-immunologically based contribute to tumor rejection (179, 180). The best approach to study these events, and to evaluate strategies to enhance their anti-tumor activity is to monitor the tumor, and the hosts' response *in situ*, i.e. without disruption of the tumor and its microenvironment. Therefore, it is anticipated that future human-SCID mouse chimeric models for evaluating cancer therapies will likely include a greater reliance upon the engraftment of fresh non-disrupted pieces of patient tumor biopsy tissues (i.e. Model #5) (Figure 1). It is also expected that refinements of this model will be made in the future that will further increase the model's potential for preclinical evaluations of treatment protocols. For example, the finding that IL-12 treatment of human lung tumor xenografts results in the upregulation of genes encoding multiple chemokines including IP-10 and MIG (Figure 2) begets the question of whether this change in gene expression also results in an increase in protein expression and ultimately in the attraction of activated T-cells which express CXCR3, i.e. the receptors for IP-10 and MIG. This could be addressed by implanting tumor bearing SCID mice with either labeled peripheral blood obtained from the patient or lymphocytes obtained from the patients' tumor draining lymph node. The homing of activated lymphocytes to the tumor could be quantified by determining the number of labeled cells in the microenvironment of IL-12 treated or control treated tumors. The impact of an increased homing of activated lymphocytes on the growth of the established tumor xenograft could be established, and the causal link of this homing with tumor suppression tested by the addition of blocking antibodies specific for the chemokine or its receptor.

It will be necessary with each of the five SCID models to begin to determine how closely the response to different treatment strategies observed in the human-SCID chimeric models reflects what is observed in patients. Results of vaccination protocols or other clinical trials based on active or adoptive immunotherapy need to be compared directly to results of the same therapeutic protocol tested in SCID mice.

All SCID models will also benefit from manipulations (either genetic or antibody depletion) that are designed to eliminate from the mouse, cells that inhibit the engraftment of human tissues, i.e. murine NK cells, granulocytes and macrophages. Once the optimal depletion protocols is established, investigators should attempt to standardize their models to diminish the variability observed from one laboratory to another.

Finally, great care must be exercised with the interpretation of the data obtained from any of the human-SCID chimeric models, particularly with respect to the potential mouse contribution to a therapy-induced alteration in tumor growth. When properly designed and with appropriate controls, these mouse models are expected to continue to provide valuable and reliable information with respect to the potential therapeutic efficacy of many different cancer immunotherapies and they will provide further insight with respect to how both innate and cognitive immune events and non-immune events (i.e. angiostatic) are coordinated and may contribute to the arrest of tumors *in vivo*.

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