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# Cancer vaccines: Uses of HLA transgenic mice compared to genetically modified mice

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## 1. ABSTRACT

Many tumor antigens have been identified that can be targeted by the immune system. Animal models that have been genetically modified to express human HLA molecules instead of their own MHC antigens have shown to be valuable in the discovery of peptides derived from tumor antigens many of which have since been used in clinical trials with varying degrees of success. Although these models are not perfect, they nonetheless allow transplantable tumor models to be developed to evaluate novel vaccination strategies that can then be applied in humans. In addition animals that have been genetically modified to "spontaneously" generate tumors that will grow within their correct environment are of greater value for studying angiogenesis, metastasis and the relationship between the immune system and tumor in a physiological setting. In this review, mice genetically modified to express HLA genes or to spontaneously develop tumors are discussed, highlighting their advantages and limitations as preclinical models for cancer immunotherapy.

## 2. INTRODUCTION

Cancer is a diverse group of diseases characterized by indefinite and uncontrolled cell proliferation. Overall one can say that tumor development is the result of a series of genetic alterations which have occurred in a multistep fashion within one cell over many years eventually leading to the emergence of a clone which no longer responds to its environment nor is controlled by the same signals that govern normal cell growth. As a consequence of such genetic changes, cell signaling and regulatory pathways in transformed cells are disturbed, giving growth advantages, modifying the micro-environment of the tumor cells and promoting escape mechanism from immunosurveillance. In addition, very early on in the disease progression, new blood vessels are created; inflammatory cells are recruited and activated in the surrounding stroma of the tumor. The interactions between tumor cells, immune cells, blood vessels and stroma cells are very complex and it is not possible to reproduce and study them in *in vitro* cell cultures. This is

**Table 1**. Comparative relevance of both models

| Model using mice transgenic for HLA molecules | Genetically engineered mice developing spontaneous tumors |
|---|---|
| Metastasize only in the lung when injected intravenously and rarely at other sites | Frequent metastasis with a distribution which often reflect those in human |
| Wrong microenvironment | Appropriate microenvironment |
| Relatively inexpensive labor | Labor intensive |
| Allow the identification of clinically relevant peptides | Peptides are specific for mice of the same strain |
| Allow the assessment of different vaccines strategies but response may differ depending on the tumor implantation. | Allow the assessment of different vaccine strategies with responses more relevant due to their correct localization. |

where mouse models of cancer, including genetically engineered mice (transgenic) and transplantable tumor models have proved indispensable tools to investigate the molecular and cellular mechanisms of tumor growth, as well as their applications in cancer research. Indeed, in spite of the various advances in the diagnosis and treatment of cancer, the conventional modalities of treatment have been unable to significantly decrease the mortality rate in patients diagnosed with advanced disease. There is therefore a great need for additional therapy that could specifically eliminate tumor cells whatever their location in the body but leave healthy tissue intact. Immunotherapy aims at manipulating/educating the patient's own immune system to arm it against tumor cells so that only transformed cells are killed. Tumor cells differ from normal cells by the accumulation and retention of many genetic alterations such as mutation, amplification, deletion, and translocation, which leads to over-production of self proteins, re-expression of proteins normally only expressed by certain organs and/or at a certain stage of the development, or production of mutated proteins, all referred to as tumor antigens. The first human tumor antigen was MAGE-1 discovered by Thierry Boon's lab (1); since then many more candidate genes have been added to the list.

All proteins produced by the cell, whether normal or abnormal, are degraded by enzymes complexes called proteasomes. This proteasomal machinery generates numerous peptides, which are then associated with newly synthesized Major Histocompatability Complex (MHC) class-I molecules and subsequently transported to the cell surface. Most nucleated cells will express MHC class-I molecules and these are consistently surveyed by $CD8^+$ T cells. Extracellular proteins are, on the other hand, first internalized by antigen presenting cells, by endocytosis or phagocytosis, encapsulated into endosomes and then degraded in the endocytic vacuoles. A phenomenon referred to as "cross-priming" can also occur where exogenous proteins are either processed by the proteasome thereby joining the class-I pathway or are first cleaved using the class-II pathway but then get transferred into the endoplasmic reticulum where they are trimmed further to fit into the groove of MHC class-I (2). The majority of peptides resulting from endocytic digestion however will bind to MHC class II molecules, which are mainly expressed by monocytes, macrophages, dendritic cells and B-cells and are surveyed by $CD4^+$ T cells.

One of the first steps needed to initiate the cascade of events that will eventually lead to an immune response is the physical interaction between the T-cell receptor (TCR) on the surface of a T lymphocyte and an antigenic peptide on the surface of an antigen presenting cell. TCRs interact with peptides "presented" by cell-surface MHC molecules. Foreign peptides presented on a cell, for example following viral infection, are recognized by T cells specific for these peptides and the cell is destroyed by cytolytic or apoptotic-induced mechanisms. Neo-antigens (abnormal self proteins arising during neoplastic transformation) may be recognized by the immune system as foreign and are potential targets for T cell mediated cellular killing. It is therefore of considerable importance to identify immunogenic and naturally (endogenously) processed MHC class-I and class-II peptides derived from tumor antigens in order to assess their potential as vaccines against cancer. It is also essential to learn more about the immune system / tumor relationship in a setting that resemble human tumor to understand the immune mechanisms of tumor rejection.

This review describes how HLA transgenic mice and genetically engineered mice developing spontaneous cancer can help answer specific questions, important for the generation of future cancer vaccines. However both models have many advantages and disadvantages and those are briefly summarized in Table 1.

## 3. TRANSPLANTABLE TUMOURS IN MICE TRANSGENIC FOR HUMAN MHC MOLECULES

There are a number of different approaches that can be adapted for the identification of new MHC class I/II-restricted target antigens for immunotherapy and many of these are in fact complimentary.

HHDII transgenic mice provide a good model for the study of HLA-A2 restricted T cells responses *in vivo* (3). These animals are "knockout" for H-2 genes, expressing instead an MHC class I molecule, consisting of the human HLA-A2.1 $\alpha$1 and $\alpha$2 domains, the murine H-2Db$\alpha$3, transmembrane and cytoplasmic class I heavy chain regions which have been covalently linked to human $\beta$2-microglobulin, allowing for HLA-A2 restricted antigens presentation within the context of the murine immune system (3). These mice have been shown to have an improved capacity to mount HLA-A2-restricted CTL responses compared with HLA-A2 transgenic mice (HLA-$A2^{+/+}$/human beta$2m^{+/+}$) that still express endogenous murine H-2b class I molecules (4). They represent a versatile animal model for CTL epitope mapping and preclinical characterization of HLA-A2-restricted CTL responses. Using HHDII transgenic mice, we have screened the Prostatic Acid Phosphatase (PAP) and HAGE proteins for immunogenic HLA-A2-derived peptides and defined

**Table 2.** List of HLA-transgenic mice (non exhaustive)

| HLA-type | Mice background | References |
|---|---|---|
| HLA-A2 H-2 class-I KO | C57BL/6 | (3) |
| HLA-A2 and HLA-DR1 H-2 class-I/class-II KO | C57BL/6 | (49) |
| HLA-A3 H-2 KO | C57BL/6 | (3) |
| HLA-DR1 | FVB/N | (50) |
| HLA-DR4/IE | C57BL/6 | (51) |
| HLA-A11/ Kb | C57BL/6 | (52) |
| HLA-B7/Kb and class-I KO | C57BL/6 | (53) |
| HLA-A27/Kb | C57BL/6 | (54) |
| HLA-A24 H-2 class-I KO | C57BL/6 | (55) |
| HLA-DQ6 and HLA-DR51 | C57BL/6 | (56) |
| HLA-B35 | C57BL/6 | (57) |

three novel epitopes (5, 6). This was confirmed and expanded by Machlenkin *et al* who were able to show that the class-I PAP-derived peptide (PAP135) pulsed onto dendritic cells could delay tumor growth in a preventative setting but also, and more importantly, could increase survival of mice bearing palpable tumor at the time of vaccination (7). Although CTL generated in HHDII mice were able to kill appropriate target cells pulsed with peptide, they were unable to kill human HLA-A2+ targets naturally expressing the antigen of interest. This is probably due to the fact that, unless $CD8^+$ T cells generated are of very high avidity for the peptide, the recognition of the target cells by $CD8^+$ T cells requires not only contact between the MHC/peptide and TCR but also between the CD8 chain and the α3 domain of the MHC class I molecule from the same species. This would explain why several *in vitro* stimulations are necessary to obtain murine $CD8^+$ T cells capable of killing antigen expressing human cancer cells (8). Still using the HHDII mice as a screening model, it is possible to assess the immunogenicity of several peptides as well as various modified peptide and even the immunogenicity of different fractions of peptides eluted from tumor cells upon the generation of CTL (9, 10). CTL generated with peptides modified at critical anchoring position, which would increase their binding affinity to specific HLA haplotypes, have been shown not only to recognize and kill target cells pulsed with the modified peptide but also recognize and kill target cells pulsed with the wild type sequence (9). Moreover HHDII mice have proved to be a useful model to study, assess, and compare the efficiency of different vaccines such as naked plasmid DNA encoding either multi-epitopes for HLA class-I and class-II peptides, or whole protein sequence either in a single agent or in a prime-boost regimen (11, 12). We have, ourselves, been able to show CTL activities of splenocytes from HHDII mice immunized with gold labeled DNA encoding the entire human mutated (at position 273) p53 protein and stimulated once *in vitro* with peptide 65, 149, 264 and 217 against RMAS/A2 cells pulsed with the same peptides with the exception of RMAS-A2 cells pulsed with the 264 peptide which were not killed (13). These results are in accordance with previously published work where peptide 264 was reported not to be naturally processed from p53 protein mutated at position 273 (14). It is therefore possible to use HHDII mice not only to identify immunogenic peptides but also and more importantly, to test whether these are endogenously processed from natural or mutated cancer protein and although the emphasis in this review is on HLA-A2 transgenic mice, HLA-A2 being the most common class-I allele, other HLA transgenic mice also exist and have also been used (Table 2). Some authors have, however, reported that the peptide repertoire resulting from the endogenous processing of the HPV derived protein by the HHDII mice may differ to that of human and caution should be taken when extrapolating the data obtained using these mice to human (15). It is therefore important to verify data obtained in these mice using PBMCs from healthy donors and/or cancer patients.

Cerundolo's group used the inability of conventional human HLA-A2 tetramers to bind to the murine CD8 molecules in the HHDII mice to show that high avidity T cells which are independent of CD8 binding can be specifically identified and isolated from these mice (16). The group then produced HLA-A2 tetramer engineered to lack the human CD8 binding capacity in order to demonstrate selective binding of these tetramers to high avidity human CTL (16). The avidity of a given T cell clone is the resulting strength of the overall interactions of MHC/peptide (binding affinity) and TCR, co-stimulatory molecules and the extracellular microenvironment. High avidity has been linked with T cell priming and its response to a given antigen. A correlation between the overall avidity and the ability of a T cell clone to recognize and kill tumor cells has also been shown (17). This avidity is generally broadly measured by the antigen dose required to induce a T cell response, where low amounts of antigen (>100 nM peptide loaded on antigen presenting cells) are required for high avidity T cell generation. These results are of great importance since cancer can be viewed as a chronic disease and TCR affinity/avidity has been shown to be the primary driving force underlying repertoire of chronic antigenic stimulation (18).

HLA class II transgenic mice were first designed to study auto-immune disease (19, 20, 21). Although, it is now accepted that $CD4^+$ T cells play a major role in initiating and maintaining $CD8^+$ immune responses (22) as well as helping in the development of memory $CD8^+$ T cells (23). More recently, Sherman's group were able to demonstrate yet another role to these cells, that of facilitating entry of $CD8^+$ T cells into the tumor microenvironment (24). Only a limited number of T-helper epitopes have been identified to date (25, 26). With this in mind, HLA class II transgenic mice expressing HLA class II molecules in the absence of murine class II molecules ($A\beta^{0/0}$) have been generated and used for the screening of immunogenic HLA class II peptide epitopes of variable chain length. HLA-$DR4^{+/+}$/$IE^{0/0}$ and HLA-DR1/$IE^{0/0}$ transgenic mice are such mice and can be used to study

human $CD4^+$ T cell immune response (27) as well as for the identification of MHC class II peptides. These mice have also recently been shown to have an enhanced efficacy over the HLA-DR1 $^{+/+}$/IAbeta$^{+/+}$mice (22, 23). Using these HLA-DR4$^{+/+}$/IE$^{0/0}$ and HLA-DR1$^{+/+}$/IE$^{0/0}$ mice we have successfully identified four novel class-II derived peptides derived from p53, PAP and HAGE proteins (5, 6, 30). We were, indeed able to show specific HLA-DR-restricted proliferation and cytokine production by splenocytes derived from p53, PAP or HAGE peptide immunized animals. Moreover mature BM-DC pre-pulsed with p53 overexpressing tumor lysate were recognized by $CD4^+$ T cells generated with the same peptide. The immunogenicity of these HLA-DR-restricted peptides was subsequently confirmed using HLA-DR4/DR1 PBMC from healthy donors, reinforcing the value of utilizing HLA transgenic mice as a pre-screening method (30).

Overall, we and others have found that HLA transgenic mice recognize the same antigenic determinants as those recognized by human T cells (31, 32) demonstrating the usefulness of such mice in finding natural tumor derived or virally derived epitopes that can be used in clinical trials.

There has been considerable interest in the generation of mice, which are transgenic for both human class I and class II molecules and knockout for the murine class I and class II molecules and some have succeeded. Indeed, the HHDII/DR1 mice are not only double transgenic for HLA-A2 and HLA-DR1 but are also double knockout meaning that these mice survive with only human MHC molecules expressed on the surfaces of their cells. Using these mice, we were able to show that specific class-I and class-II responses can be generated simultaneously after immunization with BM-DC transiently transfected with HAGE plasmid (Figure 1). Indeed splenocytes derived from HHDII/DR1 transgenic mice immunized with one round of mature syngeneic Bone-marrow derived dendritic cells (BM-DC) transfected to express the HAGE protein and stimulated once *in vitro* with either peptide HAGE126, HAGE338 or HAGE 506 were assessed for both cytotoxic responses and proliferation. Specific killing in those stimulated *in vitro* with HAGE126 could be demonstrated reaching almost 40% of total cell lysis in four out of six mice tested (Figure 1A). As a control experiment, HHDII/DR1 transgenic mice received one round of syngeneic BM-DC transfected with an empty plasmid and splenocytes were re-stimulated *in vitro* with peptide HAGE126. No cytotoxic response was achieved in both mice tested indicating that $CD8^+$ T cell responses observed earlier were actually due to the immunization with syngeneic BM-DC expressing HAGE and not the *in vitro* re-stimulation (Data not shown). Moreover, supernatants from the *in vitro* re-stimulation culture were harvested on day 3 and day 5 to measure IFNγ production by ELISA. Peptide HAGE126 allowed significant levels of peptide-specific IFNγ secretion on both days. These results were later confirmed when HHDII/DR1 mice were immunized with one round of syngeneic BM-DC pulsed with a HAGE-positive K562 cell lysate, and *in vitro* re-stimulation with HAGE126 peptide. Indeed, peptide-specific cytotoxic response of 25% was achieved in one out of two mice (Data not shown). Splenocytes re-stimulated *in vitro* with peptides HAGE338 or HAGE 506 for one week followed by $CD8^+$ depletion and a week of rest with the presence of low level of murine IL-2 in the media were then co-cultured with BM-DC matured overnight with LPS and for 2 hours the next day with PolyIC prior to the addition of the peptide. Cultures were incubated for approximately 60 hours at $37^0$C, and ($^3$H)-thymidine was added at 37kBq/well in the last 18 hours of incubation. Plates were harvested onto 96 Uni/Filter plates (Packard), the scintillation liquid (Microscint 0, Packard) was added and the plates were counted on a Top-Count counter (Packard). Specific proliferation was observed with both peptides HAGE 338 and HAGE 506-II (Figure 1B and 1C). This proliferation was shown to be HLA-DR restricted since the addition of an HLA-DR antibody (L243) could inhibit the proliferation whereas the isotype control could not. Specific release of IFNg was also observed with both peptides mainly on day 3 with however also a production of IL-5 by peptide HAGE338 but only on day 3 (data not shown).

Some groups have also been able to generate mice transgenic for class I molecules as well as a tumor antigen (33). Both of these models will prove extremely useful for investigating the role of $CD4^+$ T cells in the generation of CTL and their anti-tumoral effect. These mice will also allow the study of tolerance towards tumor antigens and subsequently ways of overcoming it; the answer of which will bring us closer to a better immunotherapeutic strategy for the treatment of human cancer.

Single and now double transgenic mice expressing only human HLA molecules can therefore be used to screen potential immunogenic epitopes, as well as assessing many different strategies for cancer vaccinations. These can include peptides, “string” of peptides or DNA/virus based vaccines administrated in a prime boost regimen with different adjuvants. Indeed, DNA based therapy using 12 peptides derived from the HER2/ErbB-2 gene was shown not only to induce peptide specific CTL responses in both HHDII mice and HLA-A2 human *in vitro* stimulated PBMC but was also capable of significantly delaying the *in vivo* growth of challenged ErbB-2-expressing tumor (EL4/HHD/neu murine thymoma) (34). These mice will also help towards the optimization of adoptive transfer therapy and the development of sensitive tools for the immune monitoring the immune system for the effects of cancer immunotherapy.

The antigen processing machinery, including specific proteasomal cleavage, TAP proteins, and chaperones, in human and mouse do however differ slightly and the overall antigen digestion and transportation may give rise to different peptide repertoire in transgenic mice and in humans. It is also known that the expression of several HLA haplotypes within one cell will induce competition between peptides thereby altering the outcome of the peptide repertoire presented by a given individual and thus rendering it different to the one found using HLA-single or double transgenic mice.

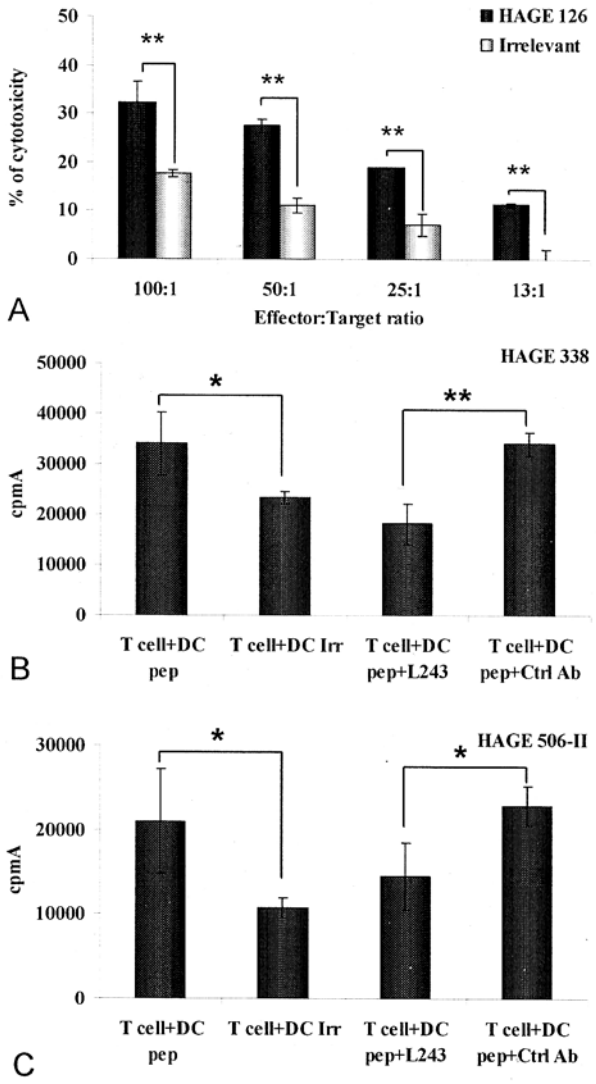


**Figure 1.** Cytotoxicity and proliferation assays were performed using splenocytes cells generated from HHDII/HLA-DR1 transgenic mice immunized with DC transiently transfected with pSHAME2a/HAGE and re-stimulated with either HAGE-derived class I peptide (126) (A), or HAGE-derived class II peptide 338 (B) or peptide 506 (C). Standard 4hrs chromium release assay was carried out after *in vitro* re-stimulation of immunized cells with immunogenic HAGE-derived class I peptides. Cytokine analysis was carried out on supernatants harvested on day 3 and 5 of the *in vitro* re-stimulation with HAGE peptide (data not shown). Specific killing can be seen against target cells pulsed with HAGE126 compared to those pulsed with an irrelevant one. Specific release of IFNg was also observed with all tested peptides mainly on day 3 with however also a production of IL-5 by peptide HAGE338 but only on day 3 (data not shown). Proliferation assays were carried out after *in vitro* re-stimulation of immunized cells with HAGE 338 (A) and HAGE 506 (B) followed by a resting period of one week with low level of murine IL-2. For the proliferation mature BM-DC pulsed with either the relevant or irrelevant peptide were co-cultured with the $CD8^-$ rested splenocytes for 60 hours with the addition of radio-active thymidine ($^3H$) during the last 18 hours. Results are expressed in counts per minute (cpm) and as means of the quadruplicate wells. Specific proliferation could be detected when the cells from immunized animals were co-cultured with DC-pulsed with the specific HAGE-derived peptides whether it be HAGE338 or HAGE506. The addition of an HLA-DR antibody in the culture was able to specifically inhibit this proliferation for both peptides whereas isotype control was not. *p<0.05, **p<0.01, ***p<0.001 are the statistical differences between HAGE-derived peptides and Irrelevant determined by unpaired Student T test.

Importantly all these studies rely on the use of transplantable tumors, which although useful to address some very precise questions, are limited when it comes to studying/targeting the local microenvironment including newly grown blood vessels feeding the tumor and the metastases. In these cases, transplantable tumors will be of no real use since most of them rarely metastasize and do not have the same relationship with their immediate surrounding due to their artificial implantation at a site which differs to the one where they originated. In an

**Table 3.** Examples of existing spontaneous tumor models in mice

| Cancer model | Genetic modifications | References |
|---|---|---|
| Breast Cancer | Mice expressing the polyomavirus middle-T oncogene driven by the mouse mammary tumor virus (MMTV) long-terminal repeat (LTR) these develop spontaneous mammary carcinomas.MUC1 transgenic mice (MUC1.Tg) expressing the human MUC1 antigen in a tissue-specific fashion similar to that in humans.MMT mice expressing the PyMT and the MUC1 antigen and develop spontaneous mammary carcinomas. | (58) (59) (60) |
| Prostate Cancer | C57BL/6 inbred TRAMP mice were generated by microinjection of a construct harboring a minimal rat probasin (PB) -426/+28 regulatory element to direct expression of the SV40 early genes (T and t antigens; Tag) to prostatic epithelium in a developmentally and hormonally regulated fashion. | (61)For a more comprehensive review on spontaneous prostate cancer refer to Abate-Shen C and Shen MM (62) |
| Pancreatic Adenocarcinoma | P53-deficient mice and TGF-α human growth hormone (hGH) fusion gene under the control of the Elastase (El) promoter (EL-TGF-a-hGH transgenic mice backcrossed on CD57BL/6. Develop rapidly growing tumors in the pancreas within 120 days after birth. | (63) (64) |
| Ovary Cancer | Cre-adenovirus/ p53+RB1 floxed recipients where more than 90% of the mice develop progressive and metastatic ovarian tumors | (65) |
| Colon adenocarcinoma | C57BL6/J mice carrying the ApcMin mutation were intercrossed with mice carrying the CDX2P 9.5-NLS Cre allele. These developed mainly colorectal tumors, with carcinomas seen in 6 of 36 (17%) of mice followed for 300 days. | (66) |
| Lung Cancers | Various models | (67) and (68) |
| Melanoma | MT-ret mice were crossed with AAD mice expressing a chimeric MHC class I composed of the α1 and α2 domains of HLA-A*0201 and the 3 domain of H2-Dd (31) to produce MT-ret+/–/AAD+/– (MT-ret/AAD) mice. More than 85% of the MT-ret/AAD mice (n = 101) displayed tumors within 4 mo after birth, and 50% of them had several evident cutaneous nodules at day 64. | (44) |

attempt to remediate to this downfall of transplantable tumors, Rosenblatt's group established a very elegant model of breast cancer metastasis where both the breast cancer cells and the bone target are of human origin (35). In this model the engrafted human bone was shown to be functional with B cells and human IgG detected in the blood stream of the mice. Moreover, the injection of a human cell line derived from a metastatic nodule of a breast cancer patient induced metastasis to the human engrafted bone and lung of the mice. This model is thereby unique in its capability of reproducing the physiological events observed in breast cancer patients with bone metastasis and it will therefore become possible to better understand osteotropism, mechanism which commonly occurs in breast and prostate cancer but for which only a mice prostate cancer model was available.

## 4. GENETICALLY ENGINEERED MICE SPONTANEOUSLY DEVELOPING TUMORS

Mouse models for cancer have existed for more than a century with the initial model being the "tumor prone inbred mice" where mice susceptible to developing tumors were bred over and over until the establishment of a strain of mice with high incidence of developing spontaneous or carcinogen-induced tumor was achieved (36). Thereafter advancement in genetic manipulation, has made it possible to genetically modify specific genes using gene targeting strategies in order to obtain mice, engineered for conditional gene expression of transforming genes, such as SV40 or polyomas, in a specific tissue (e.g the transgenic adenocarcinoma mouse prostate cancer model: TRAMP). Others have been genetically manipulated so that a cell or tissue-specific promoter will drive the expression of an oncogene known to be at the origin of tumor formation (e.g. breast cancer model: where HER2/neu gene is under the control of the mouse mammary tumor virus (MMTV) promoter/enhancer (37)). Both systems generate mice with tumors that will spontaneously develop in a particular organ and many models have been generated; Table 3 highlights many of them. These may not, however, match exactly the origin of the cell type found in human tumors; indeed, TRAMP mice, for example, develop prostate tumors of seminal origin instead of an epithelial one. However, those tumor models offer the advantage of spontaneously developing in the organ of interest. They also provide information that may be more relevant to cancer development in humans where the tumor is initiated *in vivo* by the clonal expansion from a single cell. Moreover injection of numerous tumor cells, as in the case of transplantable tumor models, may trigger inflammatory signals which may act in favor of the development of appropriate immune responses or may instead promote immuno-editing leaving behind immune-resistant subclones. By contrast, spontaneous tumors develop in a slow, progressive manner that may evoke reduced immune recognition and selection. Therefore immunotherapy against these tumors can be more difficult to raise, mimicking the challenges faced when using immunotherapy against self antigens in human tumors. It is also becoming clear that the tumor microenvironment greatly influences the outcome of immunotherapy and therefore requires careful evaluation. Recently, Wall *e. al*. (38) modified the MMTV/neu model to express at the end of the rat neu gene the dominant class-I and a class-II epitopes derived from ovalbumin. These mice were further engineered and expressed then also a dominant-negative mutant of p53 (DNp53, R172H) under the control of the whey acid protein (WAP) promoter in order to accelerate tumor formation. Using this new model, they were able to show that using the same number of adoptively transferred T cells ($CD4^+$ and $CD8^+$) both regression and non-regression responses could be obtained within the same mice, implying that these therapeutic responses were largely dictated by local, inherent properties of the tumor rather than systemic immunologic effects.

This study highlights again how important and influential the tumor microenvironment may be on the outcome of immunotherapy. Most tumor associated antigens are only expressed at certain points during tumor progression or are constitutively expressed at low level by normal cells; therefore one other major problem is the potential to develop immune tolerance, which is more likely to occur in mice developing spontaneous, slow growing tumors than transplantable tumors. Using the TRAMP model, Matteo's group highlighted that tumor antigen in spontaneously arising tumors are indeed seen as "self" antigen and vaccination strategies such as intradermal injection of peptide-pulsed DCs can have an influence on disease progression (39); while Gilboa's group (40), were able to demonstrate that an mRNA vaccine encoding tumor-associated antigens and GM-CSF could efficiently prime CTL responses in this model. However this was unable to overcome tolerance, shown to occur at the thymic level (central tolerance) as a single adoptive transfer of naive wild type splenocytes prior to the first immunization led to the specific production of CTL capable of infiltrating the tumor and reducing the prostate cancer grade. It was therefore concluded that such vaccines although very efficient in generating high avidity $CD8^+$ T cells are not sufficient to generate CTL against self antigens in animals bearing spontaneous tumors. Naive T cells are known to rapidly proliferate and differentiate into effector cells ($T_E$) shortly after encountering their appropriate antigen on antigen presenting cells *in vivo*. As the antigen is cleared, these cells will die leaving only a small fraction of residual central ($T_{CM}$) and effector ($T_{EM}$) memory T cells. Memory T cells persist a lifetime and can respond very quickly upon re-exposure to the same antigen by differentiating into $T_E$. Central ($T_{CM}$) and effector ($T_{EM}$) memory T cells which are usually discriminated by their cell surface markers, functional characteristics and migratory properties (40). Future therapy involving adoptive transfer needs to consider carefully which memory cell subtypes are more likely to survive longer *in vivo* and their therapeutic efficacy against established tumors. Using a spontaneous $gp100^+$ murine melanoma cell line (41), Restifo's group were able to show differences between the two $CD8^+$ memory subtypes in their ability to migrate to secondary lymphoid organs and not to the tumor itself as previously thought, which is required for optimal tumor eradication. This suggests that $T_{CM}$ cells might be more suited for adoptive transfer against large tumors with additional tumor-antigen vaccination (42).

Another advantage of using genetically engineered mice is the possibility of studying naturally occurring metastasis, which can be targeted after removal of the primary tumor thereby getting closer to the human development of the disease. New generations of transgenic mice have since been developed which have specific somatic mutations that are induced by tissue specific and time-controlled manner. These are better models because the surrounding stroma cells are left "untouched", i.e. non-mutated by the procedure, and therefore their influence over tumor growth can then be studied with more relevance to the development of human tumors. Many different strategies have been employed to achieve this and for a more comprehensive review on this subject one can read the very good review by Jonkers and Berns (43).

However, the main drawback of many of these spontaneous models is the difficulty in generating large cohort of mice bearing tumors at the same stage of tumor development in order to perform large experiments. Results published so far were obtained when tumors were still relatively small and the question remains whether these immuno-based therapies would still be as effective against larger tumors.

## 5. CONCLUSION

In conclusion, transgenic mice for HLA molecules are useful for the identification of naturally endogenously processed immunogenic peptides derived from tumor associated antigens and can be used to assess different vaccines strategies whether these are peptide-based vaccines with strong adjuvants, DNA/virus-based vaccines, chemotherapy alone or in combination with immunotherapy. However, these models do have their limitations in mimicking the human peptides repertoire and do not take into account the "natural" micro-environment of the origin of the tumor cells which have been transplanted, also they are not subject to the same mechanisms of tolerance and most of them do not spontaneously metastasize. This is why many researchers have turned to genetically engineered mice that "spontaneously" develop tumors within the relevant tissue, although not necessarily the same cell type as found in human cancers. One future possibility would be to develop a mouse model that is transgenic for human HLA molecules and "spontaneously" develops tumors in the appropriate tissue. Such models have started to emerge with the recent generation of an HLA-A2 transgenic mice mouse model developing spontaneous melanoma by the group of Prevost-Blondel (44). Although this model is still not ideal because the site where the first tumor developed is different to that in human i.e all the mice developed ocular melanoma first, they still carry their own MHC molecules and can still help understand why although strong CD8-mediated immune responses are detected in mice with melanoma, the disease continues to progress and metastasize ultimately leading to the death of the animal. The authors have shown that MHC and antigen expression are both tumor and animal-specific with a strong overall down regulation of both of these in visceral lesions. However, it should be noted that studying the MHC expression at varying time points can alter these results. For example in this study MHC expression was determined after culturing the tumor cells *in vitro* for a few days whilst in our hands a model of transplantable colorectal cancer (CT26) in BALB-C mice showed that overnight culturing of tumors generated from progressing animals was sufficient to restore the strong down-regulation of MHC expression which was initially observed when tumor cells had been excised from the animal and stained immediately (45). It would be of interest to assess $CD4^+$ T cell involvement and more specifically the level of Treg present in these animals since systemic ignorance, anergy and immunoediting cannot explain the growth of these cutaneous tumors.

At present time, no single mouse model will cover the full spectrum of the human disease and researchers whilst developing new models, need to bear in mind the limitations imposed by the developing tumor and its microenvironment and the differences existing between mouse and human, such as in the Toll-like receptor family (46). Toll-like receptors (TLRs) belong to a family of receptors called pattern recognition receptors (PRRs) which are used by the innate cells to recognize conserved pathogen-associated molecular patterns (PAMPs) expressed by microorganisms. To date 10 TLRs have been identified in human and 12 in mice. Their expression and stimulation have however been shown to differ between human and mice (46). Indeed TLR-2 for example which recognizes bacterial lipopeptides is present in mouse T-cells but not in human, and TLR-3 which recognizes double-stranded RNA is expressed by mouse macrophages but not human macrophages (47, 48). On the other hand TLR-4, which recognizes glycolipid of the gram-negative bacteria, although very similar in both species, is differentially regulated by LPS. In human monocytes/macrophages incubated with LPS TLR-4 expression increases whereas it is downregulated in mouse macrophages (47).

One should therefore be very careful in trying to translate any results obtained with either model into clinic. Some scientists have even started to question the entire usefulness of these models proposing to go straight to treatment.

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